University of Alberta

Introduction and spread of exotic earthworms in the boreal forest

by

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ABSTRACT

Human activity is rapidly increasing in Alberta's boreal forest and is facilitating the introduction and spread of non-native species such as earthworms. I found that earthworms were significantly more likely to occur near boat launches and roads than other locations, suggesting that bait abandonment and vehicular transport are key mechanisms of introduction. Probability of earthworm occurrence and extent of spread also increased significantly as road age increased. I used these results to create a model which indicated approximately 9% of northeastern Alberta is likely currently invaded by earthworms. However, in the next 50 years, 49% of this region is predicted to be invaded as development intensifies. Finally, mitochondrial DNA sequencing revealed that multiple introductions of *Dendrobaena octaedra* have occurred in northern Alberta. As well, genetic diversity was significantly greater at boat launches than roads, indicating that multiple introductions may be more likely to occur at those locations.

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CHAPTER 1. INTRODUCTION

1.1 Invasive Species

Non-native species are species which have become established in areas outside of their native geographic range. Non-native species which have or are likely to have a demonstrable ecological or economic impact can be defined as "invasive" species (Lockwood *et al.* 2007). Invasive species are a major cause of species extinctions and thus represent one of the leading threats to biological diversity (Wilcove *et al.* 1998; Clavero and Garcia-Berthou 2005). In the United States, non-native species are estimated to cause environmental damages and losses of almost $120 billion per year (Pimentel *et al.* 2005). Damage due to non-native species in Canada has not yet been fully quantified, although estimates for some groups are available (Dawson 2002; Colautti *et al.* 2006). For example, damage to forest plants by non-native insects and pathogens has been estimated to total $3.1 billion dollars annually, not including control or treatment costs (Dawson 2002). Given the unprecedented rate at which invasions are occurring and their tremendous costs to society, an emphasis must be placed on increasing our understanding of invasions and how to effectively manage them.

1.2 Landscape Ecology of Invasions

Landscape structure, including both habitat arrangement and type, may affect non-native species at any stage of an invasion (With 2002; Hastings *et al.* 2005). Applying landscape ecology (the study of the "effect of pattern on process" (Turner 1989)) principles to the study of biological invasions allows an understanding of how spatial pattern affects the invasion process (With 2002). In the first stage of an invasion, individuals are transported across geographic barriers to a new area (Lockwood *et al.* 2007). At this stage, landscape features such as topography may influence human land-use and thus indirectly facilitate the intentional or unintentional introduction of non-native species (With 2002). Next, individuals must establish successful populations within the new range and populations must then spread over a larger area. The spatial pattern of habitat may promote the survival, reproduction, or dispersal of non-native species during these stages (With 2002). Finally, after becoming widespread and

abundant, a non-native species may have a significant negative ecological impact. Communities in fragmented landscapes may be exceptionally vulnerable to invasion due to other anthropogenic stresses (With 2002).

Linear features may play a particularly important role in the dispersal of invasive species by acting as corridors for spread (Hastings *et al.* 2005). For example, spread of the tree pathogen *Phytophthora lateralis* depends upon road connectivity (Jules *et al.* 2002). In some cases, human activities along linear features may result in long-distance jump dispersal of non-native species to distant sites without colonization of the intervening areas. Cheatgrass (*Bromus tectorum*), for instance, was transported over long distances in North America by seed-carrying animals riding on livestock trains and trucks (Mack 1981). Despite the increasing pervasiveness of anthropogenic linear features, there has been limited research examining their effects on invasive spread at the landscape-scale (eg. Maheu-Giroux and de Blois 2007).

1.3 Invasion Genetics

Genetic and evolutionary processes may also be key determinants of an invasive species' success (Sakai *et al.* 2001; Lee 2002). Evolutionary changes, which may affect invasiveness, can occur in introduced populations due to: 1) Selective pressures that differ from those found in the species' native range (Sakai *et al.* 2001); 2) Genetic drift when small numbers of individuals are introduced (Sakai *et al.* 2001); or 3) Hybridization with other non-native or native species (Ellstrand and Schierenbeck 2000). In addition to increasing our understanding of the invasiveness of non-native populations, population genetic studies can provide information on how non-native species are introduced and spread. For example, genetic analyses revealed that the Chinese mitten crab was introduced to North America from a source in Europe rather than directly from Asia via ballast water (Hanfling *et al.* 2002). This type of information can be critical for appropriate management of invasive species.

1.4 Study Species

During the Pleistocene glaciations, earthworms were extirpated from most of Canada and the northern United States (Gates 1982). Recolonization following

deglaciation was slow and consequently much of this area remains devoid of native earthworms (James 2004). However, non-native earthworms were introduced from Europe with the arrival of European settlers in North America (Gates 1982; James 2004). In the last several decades, European earthworms have begun invading hardwood forest stands in the northeastern United States (Hale 2004). Similarly, the first records of invasion in western Canadian forests are from the mid-1980s, when aspen and pine forests in the foothills of southern Alberta were colonized by two earthworm species (Scheu and Parkinson 1994; McLean and Parkinson 1997).

As ecosystem engineers, non-native earthworms cause a variety of changes to forest ecosystems (Lavelle *et al.* 1997). Non-native earthworms can alter soil structure by reducing the thickness of organic layers and mixing organic and mineral horizons (Alban and Berry 1994; Gundale 2002; Eisenhauer *et al.* 2007). Earthworm invasion may also lead to changes in carbon and nitrogen cycling (Scheu and Parkinson 1994; Alban and Berry 1994; Bohlen *et al.* 2004). As well, earthworms have been associated with reduced microbial species richness (McLean and Parkinson 2000), local extirpation of *Botrychium mormo*, a rare fern (Gundale 2002), and decreases in abundance and diversity of understory plants (Hale *et al.* 2006).

Effects may vary depending on the ecological group to which an earthworm species belongs. Epigeic species live and feed in the leaf litter layer, and their effects are thus typically restricted to the upper soil horizons (Bouché 1977). They tend to be small-bodied, produce high numbers of cocoons (egg cases), and are capable of rapid movements. Because of their occurrence near the surface of the leaf litter layer, they are also more likely to be inadvertently transported by anthropogenic activities or non-human vertebrates than endogeic and anecic species (Terhivuo and Saura 1997). The effects of endogeic species are concentrated in the mineral soil layers, where they live and feed (Bouché 1977). Endogeic species move more slowly and have lower reproductive rates than epigeic species. Anecic species inhabit deep burrows but feed on leaf litter, and therefore cause mixing of organic material with deeper mineral soil layers. They have larger body sizes but lower dispersal and reproductive rates.

1.5 Invasions in Alberta's Boreal Forest

The boreal forest of northern Alberta is currently experiencing intense energy, forestry, agricultural, and recreational development (Schneider 2002). Associated with this development is a growing network of linear features including roads, pipelines, and seismic lines. In a 59,054 km^2 area in northeastern Alberta alone, there were more than 41,000 ha of seismic lines, 22,000 ha of pipelines, and 38,000 ha of roads on the landbase in 2002 (Schneider *et al.* 2003). These changes have had significant effects on native species such as forest songbirds (Bayne *et al.* 2005) and mammals (Dyer *et al.* 2001). However, less emphasis has been placed on determining whether human access and activity promote the introduction and spread of non-native species in forest systems (Hendrickson 2002). Non-native species that are currently present in Canada's forests include mammals, birds, insects, vascular plants, fungi, and soil invertebrates.

This study was conducted in the boreal forest of northern Alberta, between 54.4°N and 58.8°N latitude and 110.1°W and 119.3°W longitude (Figure 1.1). This area is vegetated by deciduous, mixedwood, and coniferous forests. Trembling aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), white spruce (*Picea glauca*), and jack pine (*Pinus banksiana*) are the dominant tree species in upland areas (Natural Regions Committee 2006). In lowland areas, black spruce (*Picea mariana*) and tamarack (*Larix laricina*) stands and extensive peatland complexes are most common (Alberta Environmental Protection 1994). Common shrub species include rose (*Rosa acicularis*), low-bush cranberry (*Viburnum edule*), beaked hazelnut (*Cornus cornuta*), dogwood (*Cornus stolonifera*), and saskatoon (*Amelanchier alnifolia*). Luvisols and Mesisols are the dominant soils in uplands and lowlands, respectively (Natural Regions Committee 2006). Mean summer temperature in this region is 13.7 °C and mean winter temperature is -11.9 °C (Strong and Leggatt 1992; Beckingham and Archibald 1996). Average total annual precipitation is 469 mm (Natural Regions Committee 2006).

1.6 Thesis Overview

In this thesis, I investigate the introduction and spread of non-native earthworms in the boreal forest of northern Alberta. In Chapter 2, I examine how the frequency of earthworm occurrence differs near boat launches, roads, seismic lines, lake shores, and

forest interiors. I then use this pattern of occurrence to determine which mechanisms of earthworm introduction are likely to be important in northern Alberta. In Chapter 3, I investigate the effects of road age on earthworm occurrence and spread at landscape- and local-levels. I also use this information to model the likely current and future extent of earthworm spread in northeastern Alberta. In Chapter 4, I examine the population genetic structure of *Dendrobaena octaedra*, an epigeic species, in northern Alberta and evaluate whether single versus multiple introduction events have occurred. I also assess whether anthropogenic activities influence the observed patterns of genetic diversity. In Chapter 5, I review my results and discuss their implications for management of earthworm spread in boreal forest systems.

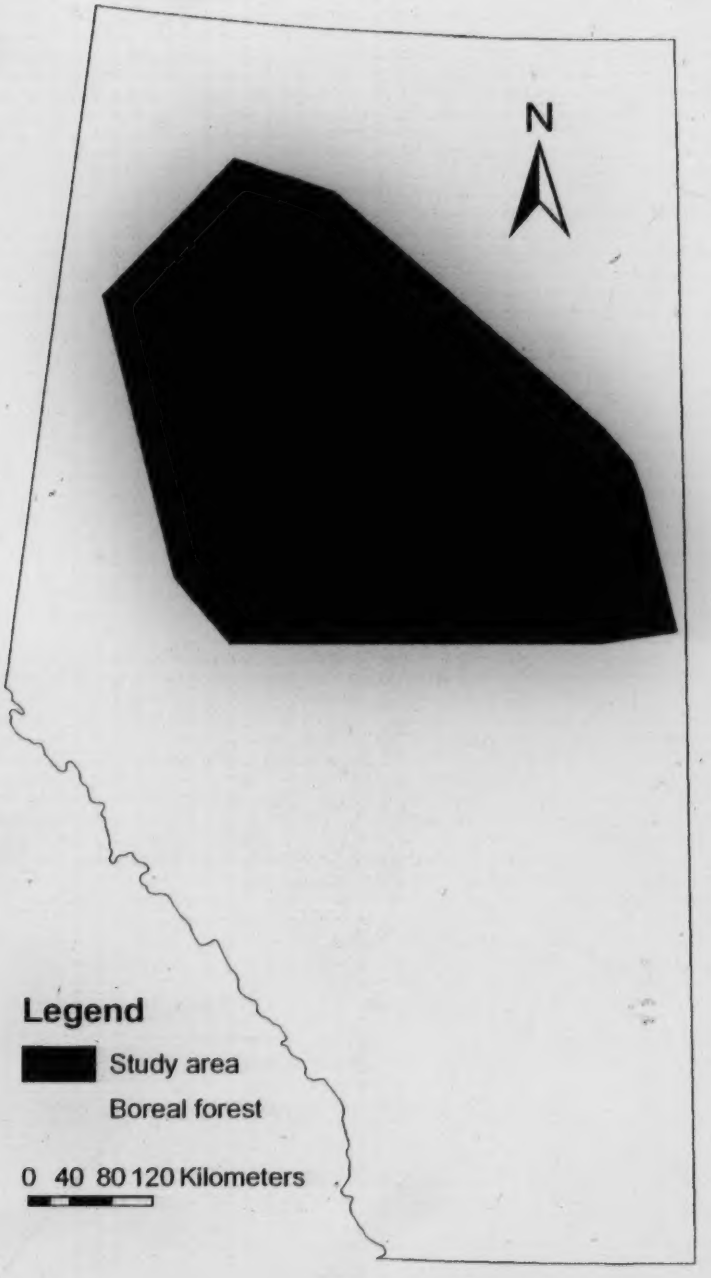

Figure 1.1. Location of study area (shown in dark grey) within the boreal forest of Alberta.

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CHAPTER 2. MECHANISMS OF EARTHWORM INTRODUCTION IN ALBERTA'S BOREAL FOREST

2.1 Introduction

Knowledge of the pathways by which non-native species are introduced to ecosystems is critical for effective management of these species (Carlton and Ruiz 2005). Although a number of studies have examined the effects of non-native earthworms in forested systems, there has been little investigation of the mechanisms by which these earthworms are introduced and spread. Because earthworms have limited active dispersal abilities (2-4m·y^{-1}), it is assumed they require some type of dispersal vector(s) to colonize systems (Marinissen and van den Bosch 1992). I propose four major mechanisms for earthworm introduction in the boreal forest: (1) Earthworm species such as *Lumbricus terrestris*, *Lumbricus rubellus*, *Aporrectodea tuberculata*, and *Aporrectodea turgida* are used as fishing bait (Reynolds 1977) and live individuals are often discarded by anglers; (2) Industrial or recreational activities may result in the transport of soil and plant materials containing cocoons or earthworms (Casson *et al.* 2002; Gundale *et al.* 2005). Cocoons may also be transported in soils picked up by tire treads of vehicles and other industrial equipment (Marinissen and van den Bosch 1992; Dymond *et al.* 1997); (3) Dispersal of earthworms and/or cocoons via non-human vertebrates has also been suggested to occur (Meijer 1972; Terhivuo 1988). While little evidence for the vertebrate dispersal hypothesis exists for earthworms, long-distance dispersal of aquatic invertebrates by waterbirds does occur (Green and Figuerola 2005); and (4) Earthworms and cocoons may be transported by waterways. Both earthworms and cocoons have been found in drift samples collected from streams, although the earthworms were generally in poor condition (Schwert and Dance 1979).

The objectives of this chapter are to measure the occurrence of earthworms in different areas in the boreal forest of northern Alberta (sites adjacent to boat launches, linear features, remote shorelines, and forest interiors) and to use these patterns to infer the mechanisms of introduction that are most likely. I hypothesized that earthworms would be present at a greater proportion of boat launches than other habitats if bait abandonment was the primary mechanism of introduction. If introduction via vehicle

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transport is common, I predicted that roads and boat launches would have similar levels of earthworm occurrence but that these habitats would have higher occurrence than remote shorelines, seismic line edges, or forest interiors. Separating the importance of bait abandonment and introduction via roads is difficult in a mensurative study as boat launches are part of the road network. However, if bait abandonment is a common introduction mechanism, I predicted that earthworm species most often used as bait (*Lumbricus terrestris*, *Lumbricus rubellus*, *Aporrectodea tuberculata*, and *Aporrectodea turgida*) should be more common near boat launches than roads (Reynolds 1977). In northern Alberta, *L. terrestris* is the main species commercially available as bait. However, some anglers dig up their own bait, in which case species that are common in yards and gardens, such as *L. rubellus*, *A. turgida*, and *A. tuberculata*, are likely to be used. If dispersal by non-human vertebrates is common, I predicted that earthworms would be present in both human accessed areas and forest interiors. Arguably however, animal dispersal could occur via vertebrate species that are better able to live in human-disturbed environments such as linear feature edges (i.e. American robin – *Turdus migratorius* or white-tailed deer - *Odocoileus virginianus*). If this were the case, I expected that earthworms would have similar levels of occurrence in edge habitat with high and low levels of human traffic (roads and boat launches versus seismic lines and remote lake shorelines). Seismic lines are 6 to 10 m wide linear features cut through forests to allow oil and gas exploration. They are used intensely for 1 to 2 months and then left to regenerate. Later, they are often used for recreational activity (i.e. all terrain vehicles) but generally are not as intensively travelled as roads. Comparison between boat launches and remote shorelines also provides an indication of the importance of water transport as a potential introduction mechanism.

2.2 Methods

2.2.1 Site Selection

This research was conducted at 17 sites in northern Alberta in July to August 2004 and June to August 2005 (Figure 2.1). I selected sites in ArcView (version 3.2, ESRI Inc., Redlands, CA, USA) using Alberta Ground Cover Classification (AGCC) and Alberta Vegetation Inventory (AVI) data obtained from Alberta Pacific Forestry

Industries. Each site was centered around a lake and contained up to six 50m transects located in 60-90 year old forest stands. Sites were selected based on the presence of suitable habitat (mature aspen or mixedwood forest) for the transects and to ensure broad-scale sampling across the study area. As well, all lakes selected were subject to sportfishing. All forest stands surveyed had not been previously used for commercial forestry or agriculture. At each site, three of the transects were located near anthropogenic features: 1) along the shore by the boat launch at a lake, 2) along the road ~2km from the boat launch, and 3) along a seismic line ~2km from the boat launch. The seismic line transects were a minimum of 300m away from roads. To control for habitat effects, each of these transects was paired with a remote transect located in a similar habitat based on ecosite classification (Beckingham and Archibald 1996): 4) across the lake along the shore, 5) 300-500m in the forest interior from the road transect, and 6) 300-500m in the forest interior from the seismic line transect. Forest interior transects were a minimum of 200m away from any anthropogenic disturbances (i.e. clearcut, wellpad, etc.) with the majority 300 to 400m distant. Transects ran parallel to the lake shore or linear feature and were located two meters from the forest edge. Five sites were missing one or two transects due to a lack of forest interior sites in certain regions that met my criteria, so the total number of transects was 17 at boat launches, 17 at far shores, 16 at roads, 16 at road forest interiors, 14 at seismic lines, and 13 at seismic line forest interiors.

2.2.2 Earthworm and Vegetation Sampling

Along each transect, I sampled for earthworms in six 0.25m^2 (50cm by 50cm) quadrats spaced at 10m intervals. Every second quadrat was offset 5m toward the forest interior in order to sample areas further from the forest edge. I removed the O_i, O_e, and O_a horizons from each quadrat and hand-sorted this material to determine the abundances of adult earthworms, juvenile earthworms, and cocoons. As with all methods of earthworm sampling, hand-sorting provides a relative estimate of abundance rather than absolute enumeration (Lawrence and Bowers 2002). However, it does produce the most accurate estimation of the number of individuals in an area for most species of earthworms (Callaham and Hendrix 1997). Earthworms were collected and preserved in

70% ethanol, and adult earthworms were later identified to species (Reynolds 1977). Because juvenile earthworms can be difficult or impossible to identify to species, I grouped them into three categories consisting of ecologically similar and/or closely related species (1) *D. octaedra* and *Dendrodrilus rubidus*; 2) *A. tuberculata* and *A. turgida*; 3) *L. rubellus* and *L. terrestris*).

In each of the earthworm sampling quadrats, I estimated the percentage cover of forbs, lichen, litter, moss, and grass based on five categories (0, none present; 1, 1-25% cover; 2, 26-50% cover; 3, 51-75% cover; 4, 76-100% cover). Organic layer thickness was estimated by taking four measurements in the quadrat with a metal meter stick and recording the average depth. As well, distance to the nearest tree, species of the tree, and distance to the nearest shrub were recorded. I also determined plant community type at the start and end of each transect using the Field Guide to Ecosites of Northern Alberta (Beckingham and Archibald 1996). This ecosystem classification system is based upon three hierarchical levels (ecosite, ecosite phase, and plant community type). Ecosites are areas sharing similar moisture and nutrient characteristics, while ecosite phases are determined based on the dominant canopy species and plant community types are based on understory species composition and abundance.

2.2.3 Statistical methods

I used a population-averaged generalized estimating equation (GEE) with a binomial distribution and logit link function within Stata to compare the occurrence of earthworms in quadrats at different locations (version 9.1, StataCorp., College Station, Texas, USA). GEEs account for a hierarchical structure within a presence-absence design and are a modification of generalized linear models (Hardin and Hilbe 2003). Thus, each quadrat occurs within a transect, with the transect as the primary sampling unit, and an exchangeable correlation is calculated for each transect. While I assumed that quadrats within a transect had a common correlation, I also used a semi-robust estimator of variance, which produces valid standard errors that are robust to any lack of independence even if the correlations within transects were not as hypothesized by the specified correlation structure. In addition to an overall test of the influence of transect type on earthworm presence, I made five specific pair-wise contrasts within the model:

1) boat launches versus far shores; 2) roads versus road forest interiors; 3) seismic lines versus seismic forest interiors; 4) boat launches versus roads; and 5) boat launches and roads versus seismic lines and far shores. Non-significant vegetation variables were removed from the analysis using a stepwise backwards elimination procedure, with P=0.15 set as the statistical rejection criterion for removing variables from the model (Hosmer and Lemeshow 2000). For the tree species variable, all coniferous trees were grouped in one category because there were very few individuals of some species. There were 558 quadrats and 93 transects included in this analysis. Because transects within a site may also exhibit a lack of independence, I performed a second analysis of earthworm presence at the transect-level in addition to the quadrat-level analysis described above. In this case, I used a GEE to account for the correlation between transects within a site. Tree community types obtained from the ecosite classifications at the start and end of the transects, rather than the tree species nearest to each quadrat, were used in the transect-level analyses. There were 93 transects and 17 sites included in this analysis. Similar analyses (at both the quadrat- and transect-levels) were used to compare the presence of only those earthworm species used for fishing bait at the road and boat launch treatments. In these cases, there were 198 quadrats, 33 transects, and 17 sites. I did not include moss or lichen cover as vegetation variables in the bait earthworm analysis because there were very few boat launch and road quadrats containing moss or lichen. Ecosites were re-categorized into three groups (aspen, aspen with another deciduous species, and mixedwood/conifer) because there were too few observations in some ecosite types (i.e. balsam poplar). All statistical tests were deemed significant at $p < 0.05$.

An extension of the Hosmer-Lemeshow test for goodness of fit, designed specifically for GEEs, was used to assess the fit of the models (Hosmer and Lemeshow 1980; Horton *et al.* 1999). This statistic is calculated by dividing the predicted values obtained from the model into groups defined by deciles, and performing a chi square test of the observed versus predicted values.

2.3 Results

I found six earthworm species at my study sites, with the most common being *D. octaedra*, an epigeic species (Tables 2.1, 2.2). The other species found were the epigeic species *D. rubidus*; one epi-endogeic species, *L. rubellus*; two endogeic species, *A. tuberculata* and *A. turgida*; and one anecic species, *L. terrestris*. Earthworm densities at different transect types ranged from $2m^{-2}$ (average across all seismic line & forest interior quadrats, including quadrats with no earthworms) to $41m^{-2}$ (average across all road quadrats). At individual transects, average densities ranged from 0 to $335m^{-2}$.

Overall, location had a significant effect on earthworm distribution in quadrats ($\chi^2 = 24.60$, df = 5, $p = 0.0002$). The transect-level covariance (correlation of quadrats within transects) was 0.67, demonstrating strong spatial autocorrelation within transects. Earthworms were significantly more likely to be present in quadrats at boat launches (64%) than far shores (21%) (Figure 2.2a; odds ratio = 6.33, $p = 0.002$). Similarly, earthworms were found at a significantly greater proportion of road quadrats (43%) than the matched forest interior quadrats (11%) (odds ratio = 5.11, $p = 0.031$). However, earthworms were not more likely to be found in quadrats at seismic lines (11%) than matched forest interiors (8%) (odds ratio = 1.60, $p = 0.710$). The proportion of boat launch quadrats with earthworms present did not differ significantly from the proportion occupied at roads (odds ratio = 2.75, $p = 0.102$), but earthworms were present at significantly more road and boat launch quadrats than seismic line and far shore quadrats (odds ratio = 6.17, $p < 0.001$). Organic layer thickness was the only vegetation variable retained in this model. Locations where earthworms were present tended to have deeper organic layers than areas where earthworms were absent (Figure 2.3; odds ratio = 1.10, $p = 0.011$), though the differences in depth were small. The goodness of fit test was not significant, indicating that model fit was adequate.

Transects within sites were also correlated (site-level covariance = 0.26), but accounting for this lack of independence produced comparable results to those obtained at the quadrat level (Figure 2.2b). Location type had a significant overall effect on earthworm distribution in transects ($\chi^2 = 52.11$, df = 5, $p < 0.001$). Earthworms were significantly more likely to be found at boat launch transects (77%) than at far shore transects (35%) (odds ratio = 5.27, $p = 0.043$), and at road transects (69%) than at

matched forest interior transects (19%) (odds ratio = 9.18, $p = 0.003$). Similarly to the quadrat level analysis, earthworm presence was not significantly different for seismic line transects (21% occupied) versus matched forest interiors (8%) (odds ratio = 2.88, $p = 0.264$) and for road transects versus boat launch transects (odds ratio = 1.36, $p = 0.686$). However, the proportion of boat launch and road transects occupied by earthworms was significantly greater than the proportion of seismic line and far shore transects occupied (odds ratio = 6.80, $p = 0.005$). Tree community type at the start of the transect was the only vegetation variable retained in this model, but it did not significantly influence earthworm occurrence. The fit of the model was adequate, as the goodness of fit test was not significant.

Earthworm species that are used as fishing bait (*L. rubellus*, *L. terrestris*, *A. tuberculata*, *A. turgida*) were significantly more likely to be found in quadrats at boat launches (30%) than at roads (1%) (Figure 2.4a; odds ratio = 0.15, $p < 0.001$) (Reynolds 1977). The transect-level covariance was 0.21. Litter depth and distance to the nearest tree were retained in this model, but did not significantly affect occurrence of bait earthworms. Similarly, bait species were significantly more likely to be found at boat launch transects (53%) than at road transects (6%) (Figure 2.4b; odds ratio = 0.03, $p = 0.001$). The site-level covariance in this analysis was 0.12. Average distance to the nearest shrub and average percentage cover of grass were retained in this model, but were not significantly related to the presence of species used as fishing bait. As well, none of these four earthworm species were found at the other transect types. The goodness of fit tests were again not significant, indicating that the models were adequate.

2.4 Discussion

2.4.1 Relative importance of hypothesized introduction mechanisms

Earthworms were most likely to be found in areas with the greatest amount of vehicle traffic (roads and boat launches). Forest interiors, forest stands adjacent to seismic lines, and forest stands along remote shores had a much lower probability of earthworm occurrence. While there are many proximate mechanisms to explain this pattern, it does suggest that human access may be the ultimate factor influencing earthworm introduction in the boreal forest of Alberta. Based on the patterns I observed,

I suggest that transport by vehicles is an important mechanism of introduction. This result is comparable to that of Gundale *et al.* (2005), who demonstrated that in Michigan, sites close to roads with a history of timber harvest were substantially more likely to be invaded by earthworms than similar sites without nearby roads or a history of timber harvest. In my study, I was able to exclude historical timber harvest as an explanation for differences in susceptibility to invasion because there was no timber harvesting in any of my sites. Conversely, in New York, distance to roads was not a significant predictor of earthworm occurrence, but it was suggested that this might have been related to low vehicle traffic on many of the roads studied (Suarez *et al.* 2006).

D. octaedra was the most common earthworm species at my sites. Because of its small size, this species is unlikely to be used as fishing bait and I believe that it, as well as the similar species *D. rubidus*, is likely being introduced by vehicles. Such epigeic species are more easily dispersed across landscapes than endogeic or anecic species due to their presence at or near the surface of the leaf litter (Terhivuo and Saura 1997), thereby increasing the probability of dispersal by human activity. In fact, in the hardwood forests of Minnesota, *D. octaedra* is typically the first to invade new systems, as it is capable of colonizing intact forest floors (Hale *et al.* 2005). As well, unlike the other species present at my sites, both *D. octaedra* and *D. rubidus* are reportedly parthenogenetic, which could allow a population to be founded following the introduction of a single individual (Gates 1974; Jaenike *et al.* 1982).

I placed the road and boat launch transects close to forest edges because earthworms may not have spread very far into the forest interior if introduction was recent. Higher probabilities of earthworm occurrence at these transects could simply be the result of forest edges being preferred habitat. However, forest edges are often drier and have increased sunlight, air temperatures, and wind velocities as compared to forest interiors (Chen *et al.* 1993; Murcia 1995). Because earthworms are dependent on high soil moisture (Edwards 2004), it seems unlikely that forest edge would be a more favorable environment for earthworms than forest interiors. Ferguson (2004) examined earthworm distribution in relation to artificially created habitat edges and found that earthworms distributed themselves randomly with respect to these artificially created edges. Furthermore, transects on the far shores and seismic lines were also associated

with edges, yet had much lower earthworm abundances which suggests that edges per se are not preferred earthworm habitat.

The probability of occurrence of all species of earthworms was similar at both boat launches and roads. However, comparisons of the earthworm assemblages at boat launches and roads revealed that earthworm species commonly used for fishing bait were significantly more likely to occur near boat launches. In fact, only one quadrat along a road contained earthworms (one adult and two juveniles) belonging to a bait species. Abandonment of bait by anglers thus also appears to be occurring in the boreal forest of Alberta.

Seismic lines experience vehicular traffic during construction, seismic exploration, and recreational use so they are not a perfect comparison to separate the relative importance of traffic and vertebrate dispersal facilitated by edges. However, earthworm occurrence near seismic lines was not significantly greater than forest interior areas, which suggests edge-adapted non-human vertebrates are relatively unimportant in earthworm introduction. If such vertebrates were facilitating earthworm spread, I would have expected that earthworms would be more common at all linear feature edges, not only at roads. Further sampling is needed in areas where seismic lines receive greater use by all-terrain vehicles. Anthropogenic edge density in northeastern Alberta is projected to more than quadruple to a density of 8km of linear features per km^2 of forest habitat over the next 100 y, due largely to seismic line construction (Schneider *et al.* 2003). Therefore, if even a small percentage of lines are sources of earthworm introductions, many forest stands could be affected.

Alternatively, the difference between occurrence of earthworms at roads and seismic lines might suggest that vehicles may not be the main introduction mechanism of earthworms. Seismic lines involve clearing the forest using a bulldozer, which creates a grassy path with relatively little soil disturbance compared to a road. The high occurrence of earthworms along roads in the boreal forest could result from earthworms being transported in soils used for road and boat launch construction, particularly if the soils are brought from regions where well- established earthworm populations already exist. In Chapter 3, however, I demonstrate that this is not likely.

I hypothesized that transport via waterways could be a mechanism of earthworm introduction. Occurrence of earthworms at far shores, however, proved to be significantly lower than occurrence at boat launches and not much greater than in forest interiors. Thus, transport of cocoons or earthworms across lakes appears to be a less frequent event than introduction by humans. This result is consistent with Suarez *et al.* (2006) who found that distance to streams was not a significant predictor of earthworm presence in New York. However, it is conceivable that transport along streams, where water flow is unidirectional, is more common than transport within lakes.

Although my study focused on potential mechanisms of introduction (i.e. the initial arrival of species in an area involving transport across some geographic barrier), the mechanisms I investigated may also be involved in the local spread of earthworms following initial introduction to the boreal forest (With 2002). Additionally, such local spread may occur due to wind dispersal of cocoons, particularly for species of earthworms such as *D. octaedra* which have light cocoons that are generally found close to the leaf litter surface (Terhivuo 1988).

2.4.2 Earthworms and habitat variables

Forest floor thickness, the only habitat variable exhibiting a significant relationship with earthworm occurrence, was substantially greater in quadrats where earthworms were present. In contrast to my results, most studies (eg. McLean and Parkinson 1997; Gundale 2002; Gundale *et al.* 2005; Hale *et al.* 2005) have found either a negative or non-existent relationship between earthworm presence and forest floor thickness. *D. octaedra*, the most common species in my study area, has not been associated with reduced thickness (Gundale 2002; Gundale *et al.* 2005; Hale *et al.* 2005), although it has had significant effects on soil structure (McLean and Parkinson 1997). Thus, my results may be indicative of earthworms' preference for areas with deeper leaf litter. Alternatively, this pattern may simply be a spurious result caused by some confounding environmental variable. The transects situated on remote shores, for example, had the shallowest forest floor but also tended to be located on steeper slopes than other transect types. These steeper slopes may have resulted in greater erosion at the remote shore transects than in other areas. In addition, if earthworm invasions have

occurred only recently, there may not have been sufficient time for large changes in habitat characteristics, such as forest floor thickness, to occur.

2.4.3 Earthworm abundance

The range of earthworm densities found at my transects (0-335·m^{-2}) was similar to densities found in forested ecosystems in other areas, although my overall averages for each treatment type (2-41·m^{-2}) such as roads were lower. This suggests that earthworms occur less frequently in the boreal forest of northern Alberta but that when earthworms are present, densities are similar to those found within other systems. For example, in a temperate-boreal transition zone in southern Sweden, beechwood forests contained 67-90 earthworms m^{-2} (Nordström and Rundgren 1973). In southern Finland, deciduous forests had earthworm densities of 167-265m^{-2} (Terhivuo 1989). Although the climate in such boreal regions is cooler, earthworm abundances in temperate forests are also comparable to those found in my study. For instance, average earthworm density in an aspen forest in Colorado was 44.44m^{-2} (Gonzalez *et al.* 2003), while in Michigan average densities were 4.9-17.7m^{-2} (Gundale *et al.* 2005). Despite these relatively similar abundance estimates, it is difficult to accurately compare earthworm densities among studies due to differences in: 1) the methods by which data are pooled to determine averages; 2) the inclusion/exclusion of areas with no earthworms in estimates; 3) the species composition in different areas; and 4) sampling techniques (eg. chemical extraction versus hand-sorting).

Because I did not hand-sort the mineral soil layers in my quadrats, the densities of endogeic and anecic species in my samples could have been much lower than the true densities of these species. To assess whether this might have substantially influenced my results, I collected 68 mineral soil samples in 2004 within the quadrats sampled that year. Only six of the samples contained earthworms (three *Aporrectodea* sp., one *L. rubellus*, and five *D. octaedra*). In all cases, the species found in the mineral soil sample was also detected in the leaf litter samples from that transect. This suggests that not sampling the mineral soil layers likely had a minimal influence on my results. In 2005, conditions were wetter throughout the sampling period, so endogeic species might have been more likely to be found in the organic horizons.

2.4.4 Conclusions

Regardless of the proximate mechanisms, the ultimate cause of earthworm introduction and spread in the boreal forest of Alberta seems to be related to human access. As such, the rapid growth of recreational and industrial activities in this region is likely to make earthworm invasions increasingly more common. As ecosystem engineers (Lavelle *et al.* 1997), earthworms may have significant impacts on the structure and function of the boreal forest, as well as on biological diversity (eg. Gundale 2002). As it is difficult to remove earthworms from systems once they become established, prevention of introduction events is desirable if such changes are to be avoided. Construction of fewer new roads would help to reduce vehicular transport of cocoons and earthworms. Adoption of regulations prohibiting abandonment of live bait could also limit introductions of epi-endogeic, endogeic and anecic species to the boreal forest.

Table 2.1. Mean relative densities (individuals extracted m^{-2}) and standard errors of adult earthworms detected in the study at boat launches, far shores, roads, road forest interiors, seismic lines, and seismic line forest interiors.

	Earthworm species					
	A. tuberculata	*A. turgida*	*D. octaedra*	*D. rubidus*	*L. rubellus*	*L. terrestris*
Transect type						
Boat launch (n=17)	0.5 ± 0.2	0.08 ± 0.1	12.0 ± 5.2	0.7 ± 0.3	1.4 ± 1.3	0.08 ± 0.1
Far shore (n=17)	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 0.8	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Road (n=16)	0.04 ± 0.04	0.0 ± 0.0	21.1 ± 11.1	0.04 ± 0.04	0.0 ± 0.0	0.0 ± 0.0
Road forest (n=16)	0.0 ± 0.0	0.0 ± 0.0	4.5 ± 2.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Seismic line (n=14)	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 1.7	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Seismic forest (n=13)	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 2.2. Mean relative densities (individuals extracted m^{-2}) and standard errors of juvenile earthworms detected in the study at boat launches, far shores, roads, road forest interiors, seismic lines, and seismic line forest interiors.

	Earthworm species		
	D. octaedra and *D. rubidus*	*Aporrectodea* species	*Lumbricus* species
Transect type			
Boat launch (n=17)	17.7 ± 7.5	2.8 ± 1.4	5.6 ± 4.2
Far shore (n=17)	1.3 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
Road (n=16)	19.8 ± 10.3	0.1 ± 0.1	0.0 ± 0.0
Road forest (n=16)	2.5 ± 2.2	0.0 ± 0.0	0.0 ± 0.0
Seismic (n=14)	1.3 ±1.3	0.0 ± 0.0	0.0 ± 0.0
Seismic forest (n=13)	1.2 ±1.2	0.0 ± 0.0	0.0 ± 0.0

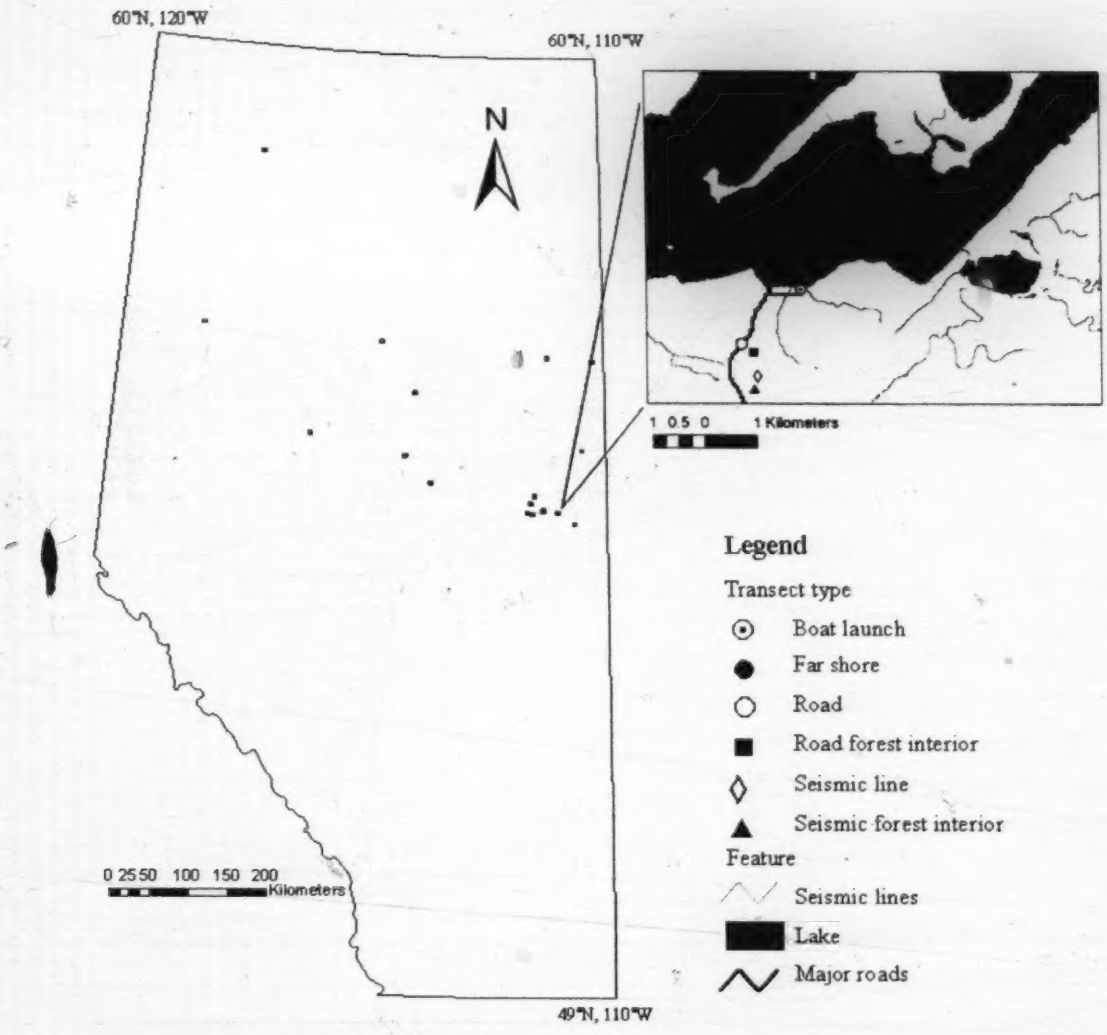

Figure 2.1. Locations of study sites (n=17) within the boreal forest of northern Alberta (shaded in gray). The layout of the transects (n=6) within a typical site is shown on the right-hand side.

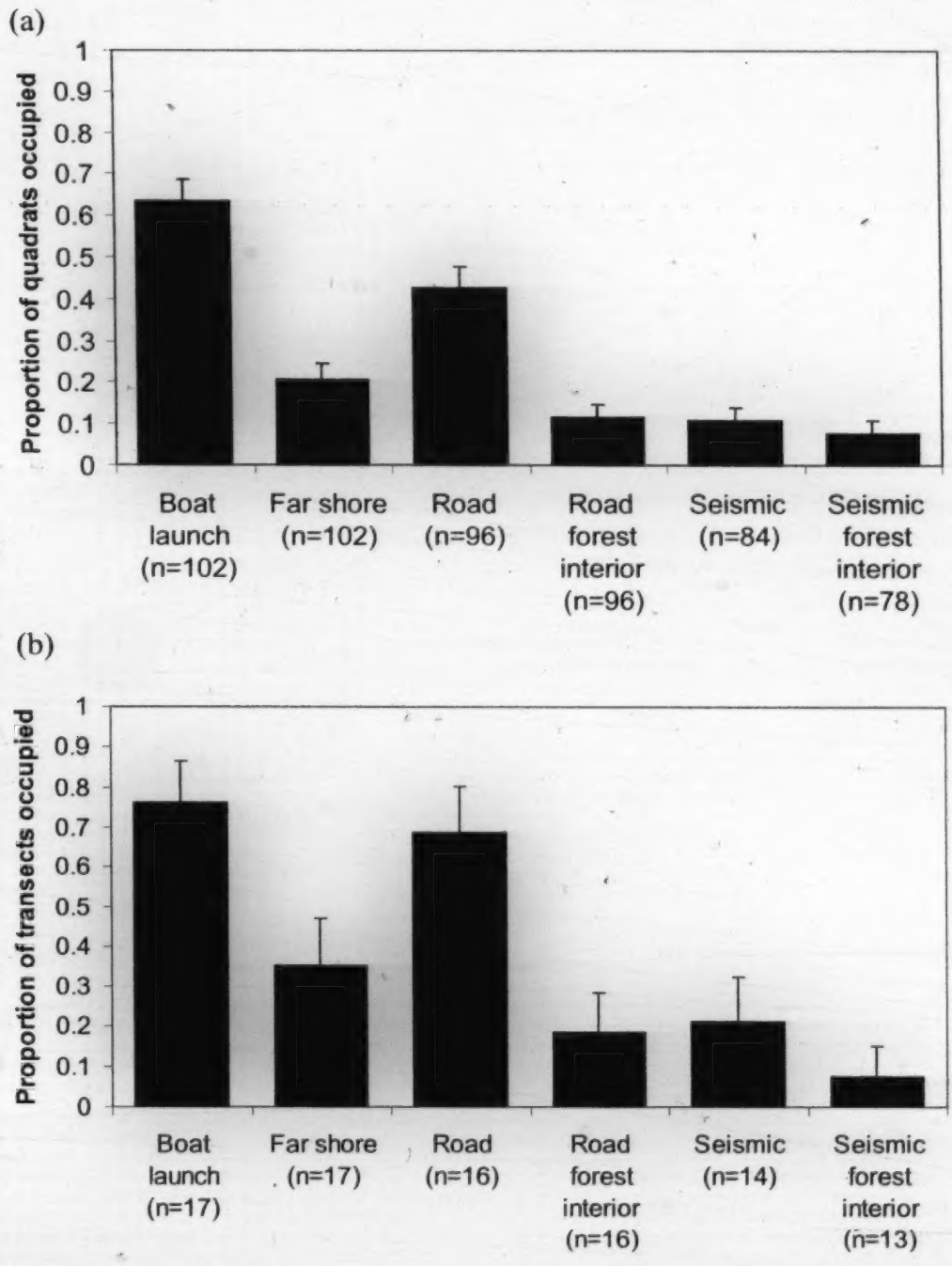

Figure 2.2. Proportion of (a) quadrats (n=558) and (b) transects (n=93) (+ SE) containing earthworms at boat launches, far shores, roads, road forest interiors, seismic lines, and seismic line forest interiors.

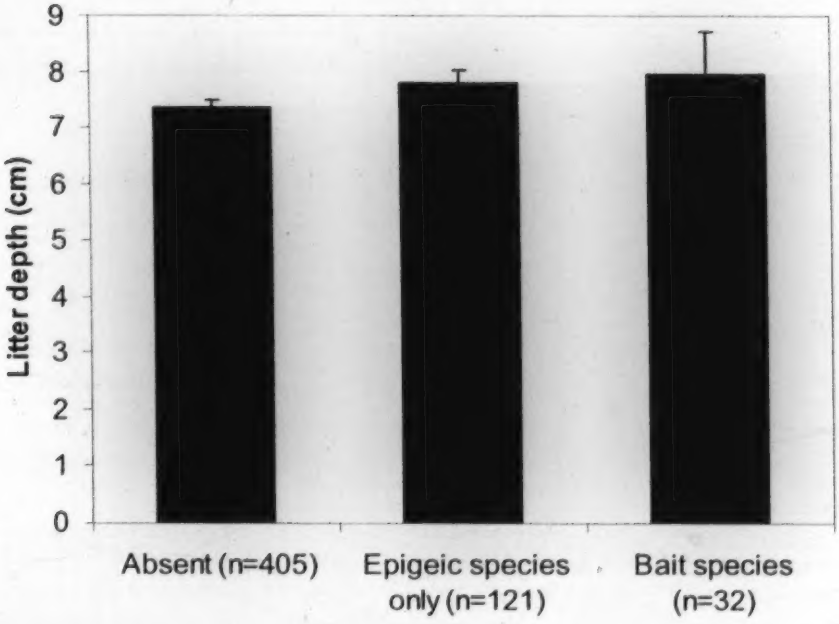

Figure 2.3. Mean (+SE) litter depth (cm) in quadrats with earthworms absent (n=405), quadrats with only epigeic species (*D. octaedra*/*D. rubidus*) present (n=121), and quadrats with bait species present (n=32).

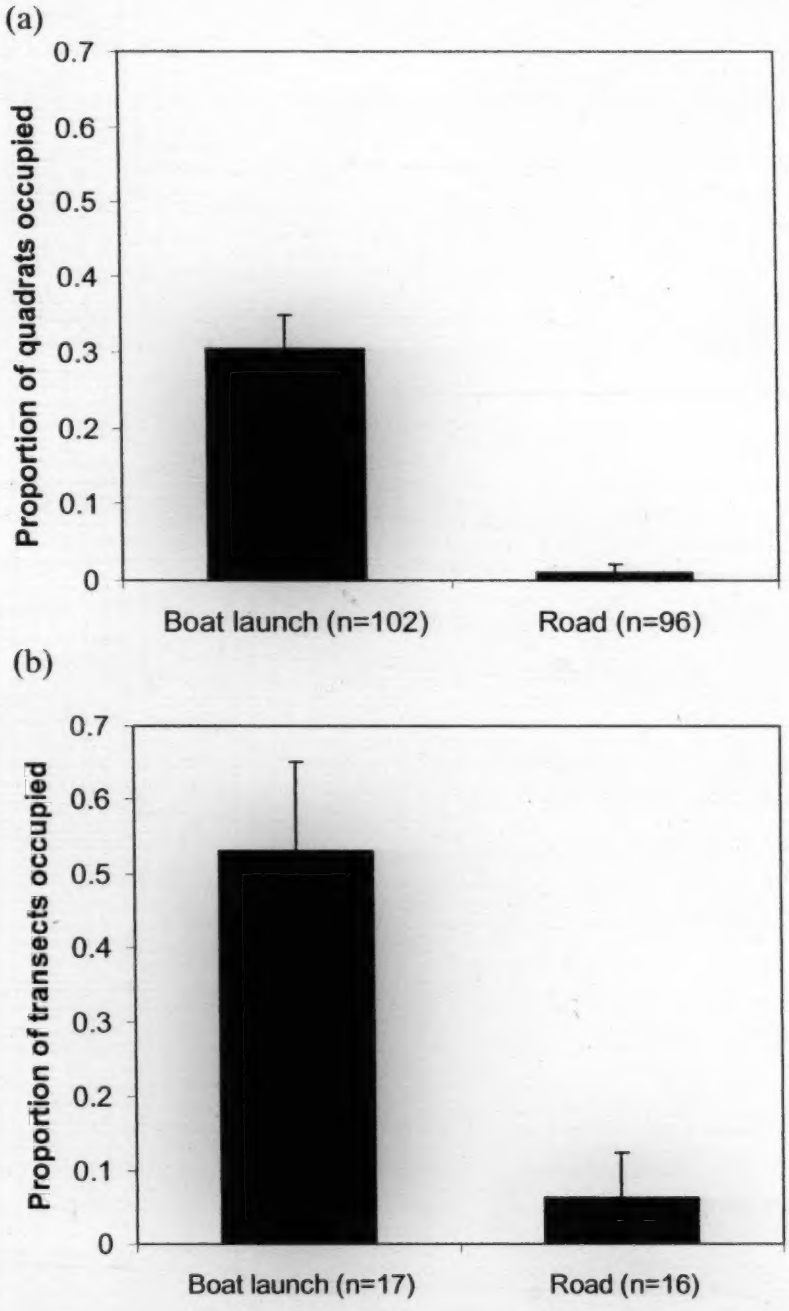

Figure 2.4. Proportion of (a) quadrats (n=198) and (b) transects (n=33) (+ SE) containing adult or juvenile bait earthworms (*A. tuberculata*, *A. turgida*, *L. rubellus*, and *L. terrestris*) at boat launches and roads.

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CHAPTER 3. ROAD HISTORY AND ITS IMPORTANCE IN EARTHWORM INVASIONS

3.1 Introduction

Roads are a key pathway in the introduction and spread of non-native species, including plants, pathogens, invertebrates, and vertebrates (Gelbard and Belnap 2003; Jules *et al.* 2002; Gundale *et al.* 2005; Brown *et al.* 2006). Higher rates of non-native species invasions are thought to occur near roads due to increases in: 1) the amount of human activity (Tyser and Worley 1992); 2) the permeability to invasions when linear features function as dispersal corridors (Tyser and Worley 1992); and 3) the availability of suitable habitats for the establishment of invasive species due to habitat modification caused by road construction and maintenance (Parendes and Jones 2000; Gelbard and Belnap 2003). Vehicles may play a particularly significant role in promoting invasions due to their ability to transport propagules over long distances (von der Lippe and Kowarik 2007).

Although roads are important in facilitating the spread of invasive species, the factors which influence the likelihood of one road versus another being colonized by non-native species are not well understood. It is believed that roads with greater human disturbance are more likely to be invaded than less disturbed roads, due to greater propagule pressure (a measure of the number of individuals introduced and number of introduction events) and increased habitat alteration associated with human activities in roaded areas (Tyser and Worley 1992; Parendes and Jones 2000; Watkins *et al.* 2003; Lockwood *et al.* 2005). For example, the cover and richness of non-native plants has been found to increase as road improvement level increases from ungraded four-wheel-drive tracks to paved roads (Gelbard and Belnap 2003). More improved roads experience greater traffic levels, habitat alteration, and frequency of use by vehicles and road maintenance equipment, all of which contributes to an increased level of disturbance (Gelbard and Belnap 2003). Similarly, roads which are subject to heavy traffic and regular maintenance experience increased rates of plant invasion as compared to abandoned roads (Parendes and Jones 2000). Another major factor that could

influence propagule pressure and amount of disturbance is the age of the road. If older roads are typically subject to higher cumulative levels of traffic and maintenance than young roads, this might result in an increase in non-native species occurrence on old roads simply due to the longer period of exposure.

In northern Alberta, linear features, including roads, pipelines, and seismic lines, currently average 1.8 km/km^2 and are projected to increase to 8 km/km^2 over the next century due to increased forestry and energy sector activity (Schneider *et al.* 2003). Given this dramatic increase in linear features, it is important to understand the risk that such features pose as invasion conduits. As discussed in Chapter 2, earthworms are one group of non-native species which appear to be introduced and spread along road networks (Dymond *et al.* 1997; Gundale *et al.* 2005; Holdsworth *et al.* 2007). A key uncertainty is whether spread along roads is more likely to occur via initial transport of earthworms or their cocoons in soil or gravel during road construction or via transport by vehicles (i.e. in tire treads or wheel wells) after the road has been built (Dymond *et al.* 1997; Gundale *et al.* 2005).

My primary objective was to assess the effects of road age on earthworm introduction and spread in Alberta's boreal forest. Specifically, I investigated earthworm introduction at the landscape-level by determining the probability of earthworm occurrence at roads of varying ages. Once a road is invaded, earthworm populations may begin to spread. Therefore, to examine the rate of spread once introduced and assess local-level spread in relation to road age, I intensively sampled earthworms at 500 m by 500 m grids. I hypothesized that earthworms would more likely be present and have spread farther at older roads as compared to newer roads. A positive relationship between road age and earthworm occurrence would suggest that earthworms are typically introduced by traffic. However, if earthworms are introduced during the construction process via the initial importation of gravel and soil, earthworm occurrence should be a function of whether or not the road fill used in construction contained earthworms. This should be independent of road age. Using these data, I then created a

spatial model to predict the current areas likely infected with earthworms and their future distribution in relation to roads in northeastern Alberta over the next 50 years.

3.2 Methods

3.2.1 Site selection

This study was conducted in the boreal forest of northern Alberta during the months of May to early August in 2005-06 (Figure 3.1). I used Alberta Ground Cover Classification (AGCC) and Alberta Vegetation Inventory (AVI) data obtained from Alberta-Pacific Forest Industries and aerial photos from Air Photo Distribution in Edmonton, Alberta to select study sites. All sites were located in mature deciduous or mixedwood stands that were adjacent to roads. Based on historical aerial photography back to 1949, I found no evidence that any of these stands had ever been cleared for agriculture or harvested. Road ages were determined by reviewing aerial photos taken between 1949 and 2005. Photographs were black and white, and had scales between 1:15000 and 1:60000. When the date that a road was built could not be narrowed down to a single year (due to gaps in coverage ranging from 1 to 16 years in length), construction was assumed to have occurred in the median year within the range of uncertainty.

In the landscape-level survey, I sampled transects at 98 roads ranging in age from 6 to 56 years old. I ensured that roads of different ages were evenly distributed across the study area. In the local-level survey, I sampled at three newer roads built after 1980 and four older roads built between 1957 and 1962. These seven sites were in locations where earthworms were known to be present along the road and where there was at least 550 m by 550 m of forest that did not contain any other linear features or human disturbances.

3.2.2 Data collection

At each site in the landscape-level survey, earthworms were sampled along a 50 m transect located parallel to the road and one to two metres from the forest edge. Six

quadrats spaced 10 m apart were sampled on each transect, with every second quadrat located 5 m further into the forest interior. In the local-level survey, I sampled earthworms on 500 m by 500 m grids, with 50 m spacing between quadrats. Each grid started approximately 5 m into the forest from the road, with the rest of the grid extending into the forest. When earthworms were not present in two consecutive quadrats in a transect running from the road to the forest interior, a quadrat 25 m from the last quadrat where earthworms were found was searched in order to more accurately map the location of the invasion front.

Earthworm and vegetation sampling at each quadrat followed the procedures in Chapter 2. However, quadrats were 0.0625 m^2 (25 cm by 25 cm) rather than 0.25 m^2 (50 cm by 50 cm). This smaller quadrat size was selected based on a pilot study I conducted, which indicated that earthworm abundance in 25 cm by 25 cm versus 50 cm by 50 cm quadrats was strongly correlated ($r^2 = 0.88$). Ecosite classification and earthworm identification were also completed as described in Chapter 2. Distance to the nearest agricultural area was calculated for all transects in the landscape-level survey using ArcGIS 9.1 (ESRI Inc. Redlands, California) and Hawth's Analysis Tools (Beyer 2004).

3.2.3 Statistical Analysis

3.2.3.1 Landscape-level

I assessed the effects of road age on earthworm occurrence by using a population-averaged generalized estimating equation (GEE) with a logit link and binomial error structure in Stata 9.1 (StataCorp, College Station, Texas), as explained in Chapter 2. In this analysis, the primary sampling unit was the transect and each quadrat occurred within a transect. I also used a semi-robust estimator of variance in case the specified error structure was inappropriate for the data. There were 588 quadrats and 98 transects in the analysis. Easting, northing, distance to agriculture, and vegetation variables were included in the global model and removed using a stepwise backwards elimination procedure if found to be non-significant. Easting, northing, and distance to agriculture were converted from meters to kilometers. The statistical rejection criterion for

removing a variable was P=0.15 (Hosmer and Lemeshow 2000). An extension of the Hosmer-Lemeshow test for goodness of fit, designed specifically for GEEs, was used to assess the fit of the model, as described in Chapter 2 (Hosmer and Lemeshow 1980; Horton *et al.* 1999).

3.2.3.2 Local-level grids

I created maps of earthworm abundance at each grid using ordinary kriging in ArcGIS 9.1. Ordinary kriging is an interpolation technique that uses known values obtained at sampling points to predict unknown values at other points within the sampling area (Isaaks and Srivastava 1989). Zero-inflated negative binomial regression was also used to test the effects of distance from the road, age class of the road, and the interaction between age class and distance on earthworm abundance and occurrence within Stata 9.1. Zero-inflated regressions use a two-part modeling approach, consisting of a binary outcome model which models the probability of obtaining a zero count and a truncated count model which models the non-zero counts (Cameron and Trivedi 1998). This type of regression is suitable for data that displays overdispersion and a high incidence of zero counts. To account for the probable lack of independence of quadrats within sites, a robust clustering approach was employed. This technique uses a variance estimator to adjust standard errors, and thereby accounts for within-site correlations (Rogers 1993). Non-significant vegetation variables were removed using a stepwise backwards elimination procedure, with $p = 0.15$ as the statistical rejection criterion (Hosmer and Lemeshow 2000). I assessed goodness of fit using a chi square analysis of predicted versus observed values, and compared the fit with that obtained from poisson, negative binomial, and zero-inflated poisson regressions. Finally, I calculated the approximate average rate of earthworm spread from the roads by dividing the distance of the invasion front from each road by the age of the road.

3.2.4 Spatial Modeling of Future Earthworm Distribution

I used ArcGIS 9.1 to generate a model of current and future earthworm distribution in the Alberta Pacific Forestry Industries Forest Management Area (Al-Pac

FMA), a 59,054 km^2 area in northeastern Alberta. There is little data available on the age structure of the road network in northern Alberta, although I was able to obtain the ages of some roads from the Mistakiis Institute. Ages were assigned to the remaining roads based on the average age of the nearest wells and cutblocks, because much of the road network was built to access these features. To create a future road network with a realistic road density, I used ALCES® (A Landscape Cumulative Effects Simulator) to obtain projected road densities over the next 50 years (see Schneider *et al.* (2003) for details of the model). Road networks from the 15% of townships with the highest road densities were then randomly added to the existing network in ArcGIS until the overall road density was equal to the projected ALCES® estimate for each decade.

The "convert paths to points" tool in Hawth's Analysis Tools for ArcGIS (Beyer 2004) was then used to generate a point every 10 m along the road network. These points were randomly infected at a rate of 1.03 % per year, excluding the year in which the road was built. This infection rate was obtained from the slope of a linear regression of my earthworm occurrence data in relation to road age. Buffers were then created around each infected point in order to determine the areal extent of earthworms in 50 years, assuming a spread rate of 10 m per year. To determine the total area of forest likely to be invaded, I intersected this buffered layer with a layer containing forest habitat suitable for invasion. Suitable habitat included all forest types except those dominated by black spruce (*Picea mariana*) or tamarack (*Larix laricina*), as such forests have highly acidic soils and are thus less likely to be colonized by earthworms (Edwards and Bohlen 1996).

3.3 Results

3.3.1 Landscape-level

Of the 588 quadrats sampled, 204 (35%) had earthworms present. Similarly, earthworms occurred along 44 out of 98 transects (45%). Densities within quadrats ranged from 0 to 1040 earthworms/m^2, with an average of 42 earthworms/m^2. The most common species at my sites was *D. octaedra* (99.8% of adults). *D. rubidus* and

Aporrectodea tuberculata were also present. Most juveniles were *D. octaedra* or *D. rubidus*, although there were also four *Aporrectodea* sp. individuals.

Road age had a significant effect on earthworm occurrence at the landscape-level, with older roads being more likely to have earthworms present than younger roads (odds ratio = 1.07, $p = 0.001$; Figure 3.2). The correlation of quadrats within transects was 0.67, indicating that strong spatial autocorrelation existed within transects. Distance to the nearest shrub (odds ratio = 0.994, $p = 0.036$), distance to agriculture (odds ratio = 1.02, $p = 0.021$), easting (odds ratio = 0.993, $p < 0.001$), and northing (odds ratio = 0.988, $p < 0.001$) also had significant effects on earthworm occurrence. Distance to the nearest tree was retained in the model as well, but did not significantly influence earthworm presence. The goodness of fit test was not significant ($\chi^2 = 13.2$, $p = 0.11$), which suggests that the fit of the model was adequate. I also used standard logistic regression to conduct a similar analysis of earthworm occurrence at the transect-level, rather than within quadrats, which produced similar results.

3.3.2 Local-level grids

343 out of 847 quadrats (40.5%) on the local-level grids were occupied by earthworms. Earthworm densities in the quadrats ranged from 0/m^2 to 1200/m^2, with an average density of 81 earthworms/m^2. *D. octaedra* was the most common species (96.9% of adults), followed by *D. rubidus* (3.0% of adults), and then by *L. rubellus* (0.0005% of adults). All juveniles were *D. octaedra* or *D. rubidus*.

The kriged maps for the older roads showed that earthworms had spread to the back of the grid at each site (Figure 3.3). For the newer roads, the maps showed that earthworms had spread less than 100 m from the road in each case (Figure 3.4). Earthworm abundance decreased significantly as distance from the road increased (incidence rate ratio = 0.9989, $p = 0.001$). However, age class and the interaction between distance and age class did not significantly affect earthworm abundance. Tree distance was the only habitat variable which significantly influenced earthworm abundance (incidence rate ratio = 1.0015, $p = 0.01$). The probability of earthworms

being absent also increased significantly as distance from the road increased (odds ratio = 1.0491, $p = 0.0001$). As well, the distance and age class interaction had a significant effect on earthworm absence, with newer roads being more likely to have earthworms absent as distance increased than older roads (odds ratio = 0.9596, $p = 0.001$). Age class did not significantly affect earthworm absence. No vegetation variables significantly affected earthworm absence, although distance to the nearest shrub and percentage cover of forbs were retained in the model. The goodness of fit test showed that zero-inflated negative binomial regression produced predictions that agreed more closely with the observed data than those produced by poisson, negative binomial, or zero-inflated poisson regressions. The average rate of earthworm spread at the younger roads was 1.8 m/year. Because earthworms were present at the farthest points sampled at the older roads, invasion fronts could not be identified and I was unable to calculate spread rates for those sites.

3.3.3 Modeling

Total road length in the Al-Pac FMA is currently 22,068 km. The total area of suitable forest habitat for earthworms within the FMA was 24,447.6 km^2. Using current road densities and estimated road ages, my model predicts that 2221.9 km^2 (9.09%) of this area is currently invaded by earthworms (Figure 3.5). If rates of road development occur as predicted by projections from ALCES® (Figure 3.6), I expect that there will be 54,477 km of roads built over the next 50 years. According to my model, this will result in 12,044.6 km^2 (49%) of the Al-Pac FMA being invaded by earthworms within 50 years (Figure 3.7).

3.4 Discussion

3.4.1 Landscape-level occurrence

In northern Alberta, road age appears to strongly affect the occurrence of non-native earthworms, with older roads being significantly more likely to have earthworms present than younger roads. Older roads probably have experienced a greater cumulative

amount of vehicular traffic and other disturbances such as road maintenance than roads constructed more recently. This could result in greater propagule pressure and an increased probability of earthworm occurrence along older roads. My finding is comparable to previous studies which found that invasive plant species richness increased with time since human settlement in American states (McKinney 2001) and protected areas (McKinney 2002).

Earthworm presence was also significantly influenced by site location. Earthworms were more likely to occur towards the south and east boundaries of my study area, where anthropogenic activities have historically been more intense. Therefore, there has probably been more opportunity for earthworm introduction in these areas over time than in the northwest. Agriculture, which is also concentrated in the south and east, significantly affected earthworm occurrence as well. This is consistent with a study which found that agricultural fields appear to act as major sources of non-native earthworm introduction in New York (Suarez *et al.* 2006). Earthworms and cocoons are likely transported to farms on the tires of trucks or agricultural machinery, and in potted plants (Marinissen and van den Bosch 1992; Suarez *et al.* 2006). Earthworms can attain high densities in agricultural clearings and thereby act as important source populations for invasion of nearby areas (Suarez *et al.* 2006).

Shrub distance was the only habitat-related variable that was associated with earthworm occurrence. Earthworms were more likely to occur in quadrats closer to shrubs, a result which may be due to the confounding effects of other unmeasured variables such as litter quality, nutrient availability, temperature, or moisture that might vary with distance to the nearest shrub. The lack of a relationship between earthworm occurrence and the other habitat variables may be related to the fact that the endogeic and anecic species which typically cause the most rapid changes in litter depth and other habitat characteristics were not present at the majority of my sites (Gundale 2002; Hale *et al.* 2005). These species generally invade after litter-dwelling epigeic species have established (Hale *et al.* 2005), and in northern Alberta currently appear to be localized largely near boat launches as discussed in Chapter 2.

Other characteristics of roads appear to be important determinants of invasive plant occurrence, and thus may also affect earthworm establishment. First, higher levels of road improvement are associated with increased cover of non-native plant species (Gelbard and Belnap 2003). Because most of the accessible roads in my study area are gravel, I was unable to test whether road improvement affects earthworm occurrence. Secondly, high traffic can result in higher rates of non-native plant invasions (Parendes and Jones 2000). I estimated traffic levels (none, moderate, or high) at 91 of my sites but found no relationship with earthworm occurrence. However, traffic levels obtained at a single point in time may be a poor measure of actual use because use of many roads in this region is highly variable over time. As well, this approximate rating system may not have been precise enough to detect traffic effects, and a more accurate count of the actual number of vehicles traveling along the roads could have revealed stronger traffic effects.

3.4.2 Local spread

Because the processes governing dispersal of invasive species may differ between scales, I examined spread at the local-level in addition to the landscape-level (Pauchard and Shea 2006). Road age also appears to strongly influence local spread of earthworms as spread had occurred over significantly greater distances from older roads as compared to younger roads in my study sites. The opportunity for earthworm introduction via vehicular transport would likely have occurred much earlier in areas with older roads than in those with roads built more recently. This means that there should have been more time for earthworms to spread, resulting in populations that extend over a larger area at such locations.

Earthworm occurrence and abundance in the local-level survey were also affected by distance from roads, with fewer earthworms occurring as distance increased. This result is consistent with previous research that examined earthworm spread at shorter distances from roads in Alberta (Dymond *et al.* 1997). In contrast to this, in hardwood forests in the northeastern United States, road presence appears to be more appropriate as a coarse-scale rather than fine-scale predictor of earthworm invasion (Suarez *et al.* 2006;

Holdsworth *et al.* 2007). This may be related to the fact that earthworm invasions in the northeastern United States likely began earlier than invasions in Alberta (100 years versus 50-60 years), and consequently earthworms would be expected to have dispersed farther from initial introduction sites in the hardwood forests (Holdsworth *et al.* 2007). Initial introductions thus appear to have occurred along the roads at my study sites, with subsequent spread occurring towards forest interiors. Cocoons of *D. octaedra* and *D. rubidus*, the most common species in my samples, are particularly likely to be transported by vehicles because they are produced in large quantities via parthenogenesis, and are found in the upper leaf litter layers (Gates 1974; Jaenike *et al.* 1982; Dymond *et al.* 1997; Terhivuo and Saura 1997).

Distance to the nearest tree was the only other variable which had a significant effect on earthworm abundance. This relationship may be due to the influence of a confounding variable that was not measured, such as soil moisture levels. Soil moisture often has a strong positive influence on earthworm abundance (Edwards 2004), but it may be reduced near trees due to the interception of precipitation by the tree canopy. This could then result in decreased earthworm abundance.

Information on the spread rate and areal extent of an invasive species is crucial for the development of appropriate management strategies (Abbott 2006). The average spread rate at the younger road sites (1.8 m/year) is likely an underestimation of the actual rate of earthworm spread because initial introduction of earthworms may often occur several years or more after road construction. No spread rates could be calculated for the older roads, which had an average age of 46 years, because earthworms had spread to the farthest points sampled. This suggests that the average spread rate at those roads was greater than 10 m/year, particularly if initial introduction did not occur soon after construction. Although I was only able to calculate approximate rates of earthworm spread from my data, my results suggest that the rate of earthworm population expansion in the boreal forest is similar to or higher than the rates reported in other areas (~5-10 m/year) (Marinissen and van den Bosch 1992; Hale 2004).

3.4.3 Modeling

My model of earthworm spread in the Al-Pac FMA of northeastern Alberta suggested that approximately 49% of suitable forest habitat will be occupied by earthworms in the next 50 years. This model was based upon a number of assumptions that may have affected this estimate of the future extent of earthworm invasion. First, I assumed that there were no barriers present that could prevent earthworm spread within the buffered areas. Some suitable forest habitats within the buffers may have been isolated from previously infected stands by, for instance, black spruce or tamarack stands such that earthworms would be unable to spread into them. Secondly, introduction rates may vary depending on the level of road improvement or traffic levels as discussed previously. As well, introduction may occur along waterways, or near other anthropogenic features such as pipelines or seismic lines (Schwert and Dance 1979). Thirdly, I set 10 m/year as the spread rate in my model but my local-level data suggest that spread may occur more quickly than 10 m/year. Estimates using data from multiple years are necessary. Fourthly, the age structure or future density of the road network may be incorrect. Finally, I assumed that roads were placed randomly on the landscape, although most future roads will likely be built in specific locations to allow access for resource extraction.

3.4.4 Conclusions and Implications

Road age had a strong influence on earthworm occurrence and spread in northern Alberta. Although the effects of road age on invasions have not been examined previously, my findings are consistent with studies on non-native plants which demonstrate the importance of increased disturbance levels and greater propagule pressure in promoting species invasions near roads (eg. Gelbard and Belnap 2002). Consequently, effects of road age similar to those found in my study might be expected for other groups of invasive species, such as plants, and should be investigated further.

Reducing the number of new roads being constructed may help to decrease earthworm introduction and spread. However, my study suggests earthworms are more

often introduced by vehicle traffic than during initial importation of gravel and soil during construction. Therefore, earthworm introductions may also be reduced by restricting the amount of traffic on roads or by reclaiming temporary roads whenever possible. The risks posed by earthworms to boreal forest hydrology, carbon dynamics, and biodiversity could be substantial. The very large area of northern Alberta that earthworms are likely to invade in the near future if current rates of development persist, make it important to try to slow the introduction rate of this ecosystem engineer.

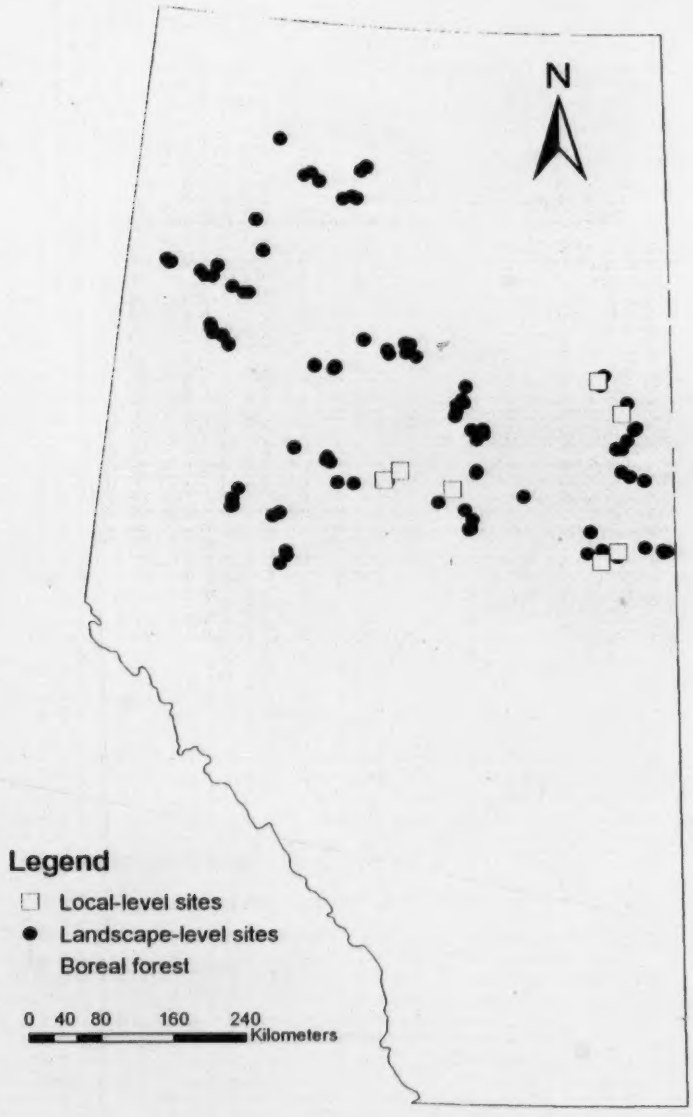

Figure 3.1. Locations of study sites within the boreal forest of northern Alberta. Black circles represent landscape-level sites and white squares represent local-level sites.

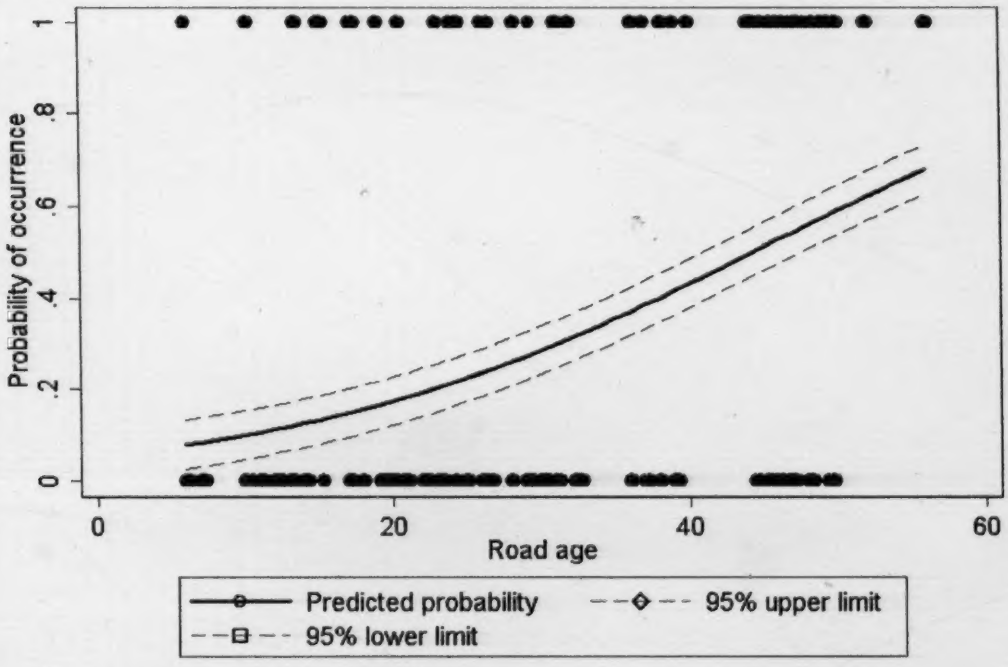

Figure 3.2. Regression plot of predicted probability of earthworm occurrence (solid line) in relation to road age. Dotted lines represent 95% confidence intervals and black dots represent actual presences and absences. Other model covariates are held at their mean values.

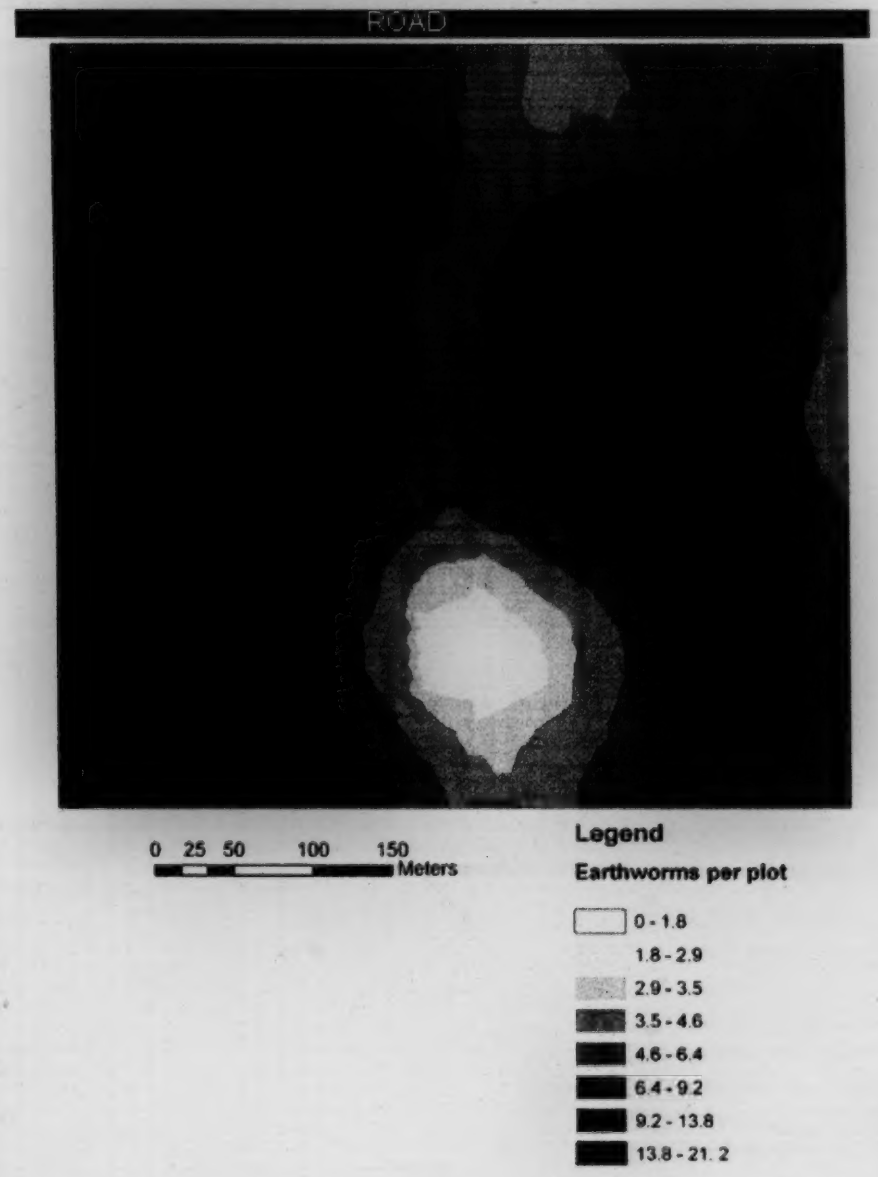

Figure 3.3. Kriged map of earthworm occurrence at a typical older road site. The legend shows the predicted number of earthworms per 0.0625m^2 quadrat.

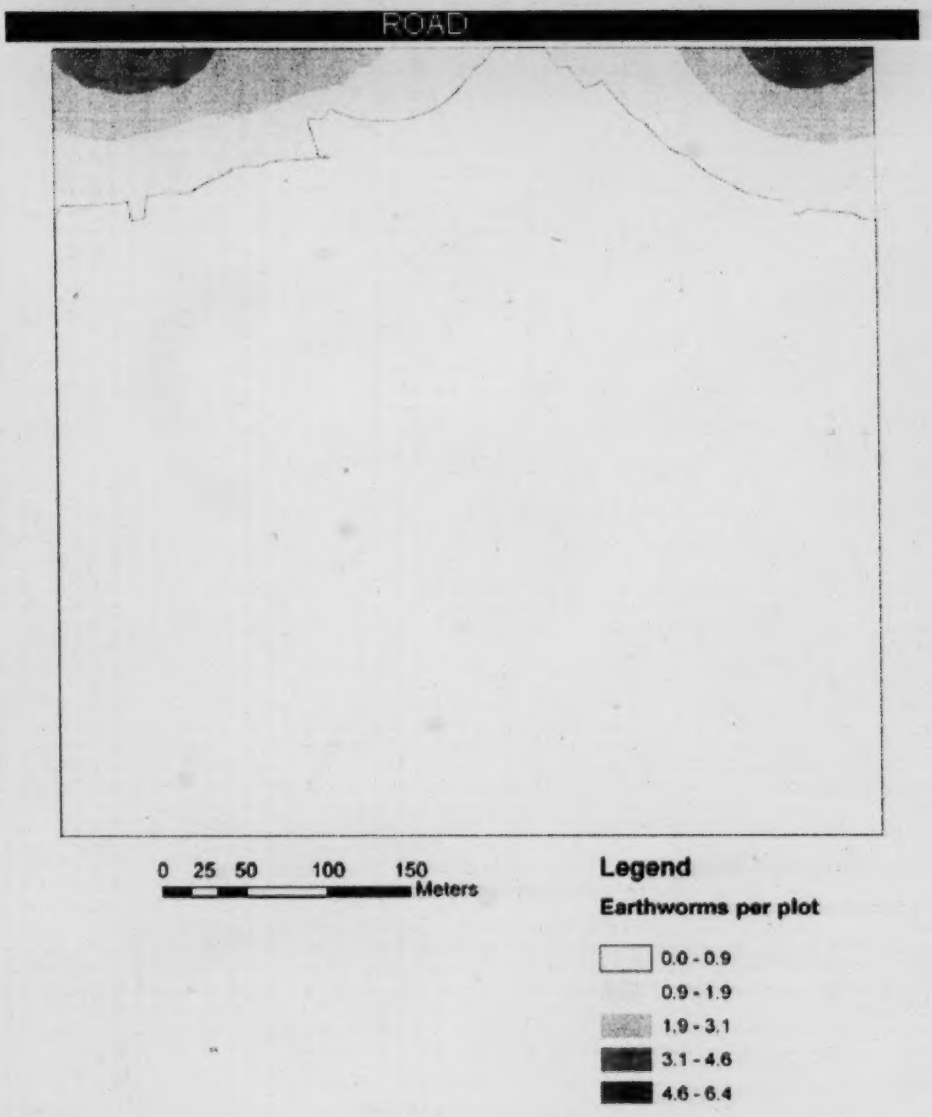

Figure 3.4. Kriged map of earthworm occurrence at a typical newer road site. The legend shows the predicted number of earthworms per 0.0625m^2 quadrat.

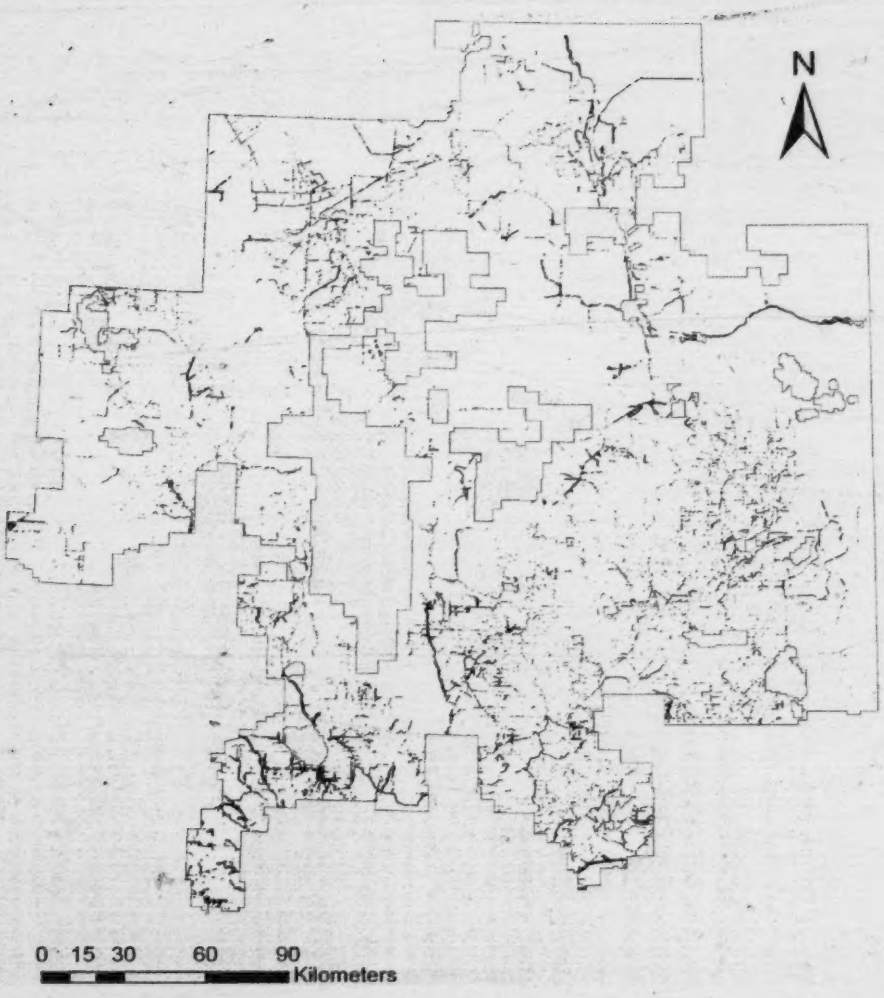

Figure 3.5. Projected current extent of earthworms in the Al-Pac FMA of northeastern Alberta. Areas invaded by earthworms are shown in black and the Al-Pac FMA is shown in white.

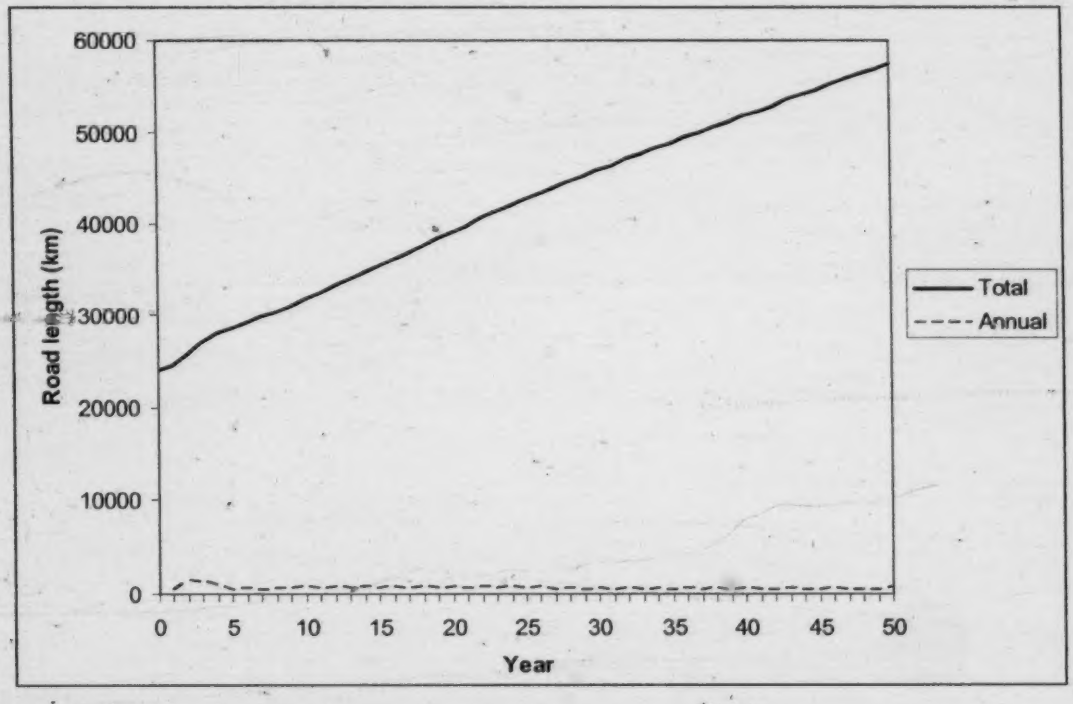

Figure 3.6. Total and annual lengths of roads built (in kilometers) within the Al-Pac FMA over the next 50 years, based on projections from ALCES®.

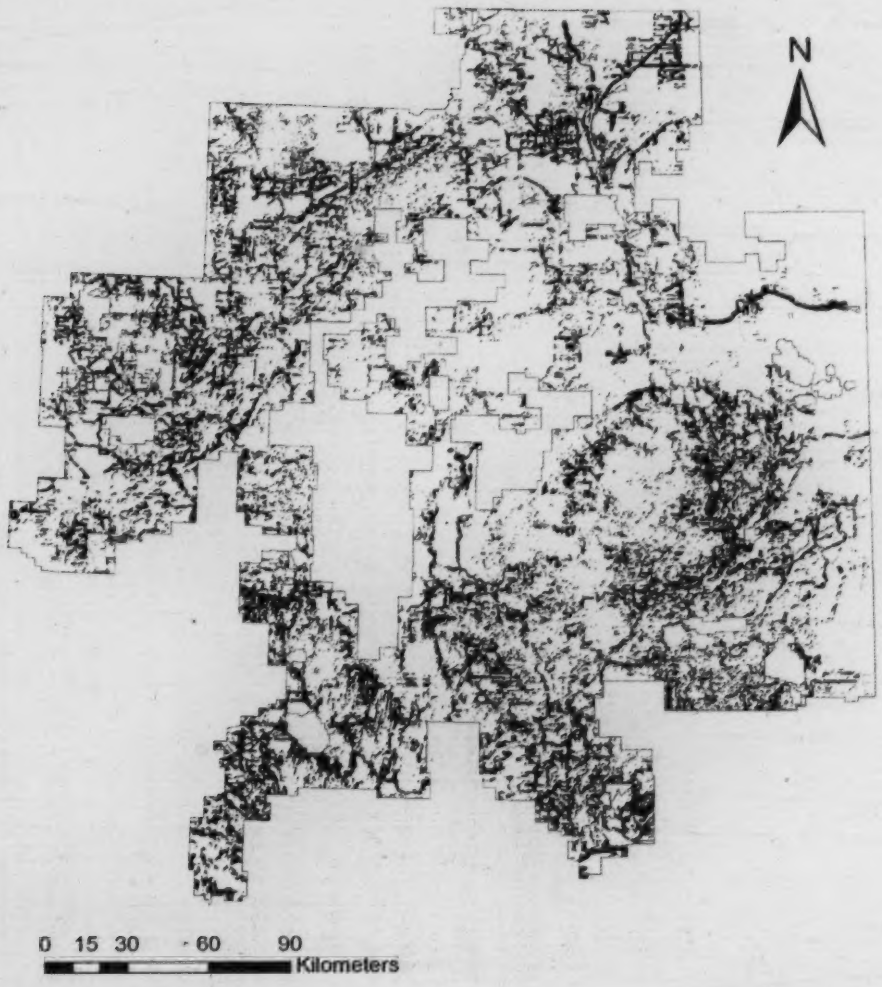

Figure 3.7. Predicted future extent of earthworm occurrence within the Al-Pac FMA in 50 years. Areas invaded by earthworms are indicated in black and the Al-Pac FMA is shown in white.

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CHAPTER 4. POPULATION GENETIC STRUCTURE OF *DENDROBAENA OCTAEDRA*: INSIGHTS INTO INTRODUCTION MECHANISMS

4.1 Introduction

The population genetic structure of non-native species can have a strong influence on invasiveness (Sakai *et al.* 2001; Lee 2002). Populations of non-native species that have been founded by a single introduction event often have reduced genetic variation relative to their source populations (Barrett and Kohn 1991; Allendorf and Lundquist 2003). In many situations, this results in a decreased ability to adapt to the local environment, potentially reducing the risk of spread (Sakai *et al.* 2001). In some cases however, multiple introductions of individuals from a number of different sources occurs into the same non-native population. Multiple introductions can produce greater genetic diversity in non-native populations than in native populations which can facilitate rapid adaptation and expansion in the species' new range (Kolbe *et al.* 2004). As such, knowledge of whether populations have been established via single or multiple introduction events is crucial to understanding the ecology of invasive species and designing appropriate management.

Understanding population genetic structure and the occurrence of single versus multiple introductions can also provide critical information on the mechanisms by which invasive species are introduced and spread. For instance, population genetic structure of the invasive quagga mussel (*Dreissena bugensis*) revealed that long-distance jump dispersal by boaters, rather than spread via gradual diffusion, was the most important factor in its spread (Wilson *et al.* 1999). In the brown alga, *Undaria pinnatifida*, genetic structuring suggested a more complicated pattern of invasion, in which the mechanisms involved in its spread varied across the invaded area (Voisin *et al.* 2005). Aquaculture appeared to be a major mechanism involved in the spread of this species in Europe, whereas spread by maritime traffic was likely more important in Australasia.

D. octaedra, as discussed in the previous chapters, is an earthworm species that is not native to the boreal forest of northern Alberta. Although native to Europe, this

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generalist species is now widespread in the northern hemisphere and has been introduced to the United States, Mexico, Asia, and across Canada (Reynolds 1977). *D. octaedra* reproduces via apomictic parthenogenesis, in which eggs are produced by mitosis rather than meiosis (Omodeo 1955; Casellato and Rodighiero 1972). Offspring are thus genetic copies of their parent, and a single individual is capable of establishing an invasive population. In this chapter, my interest was in determining whether invaded areas were established by single or multiple introduction events from one or multiple sources. These sources might be located either outside of the boreal forest of northern Alberta (eg. in the agricultural area to the south or east), or within the system (eg. at boat launches or roads that were colonized earlier). Given that *D. octaedra* are parthenogenetic, it is possible that a single haplotype exists in the boreal forest and is simply spreading within the system (Facon *et al.* 2003). Alternatively, each invaded population may have been founded by a single invader but these invaders could each have been from different source populations. If this is the case, new introduction sites might be more likely to contain unique haplotypes (Bohonak *et al.* 2001). Finally, if populations are founded by multiple invaders, from either single or multiple sources, multiple haplotypes should be detected within each population (Bohonak *et al.* 2001). Populations founded by multiple invaders may require more intensive management due to their increased potential for adaptive evolution.

The mechanisms facilitating dispersal of an invasive species are likely to have a strong influence on population genetic structure and occurrence of single versus multiple introduction events. Therefore, in order to obtain a greater understanding of earthworm spread in the boreal forest, I examined genetic variation of populations at boat launches and roads. I focused on populations near these types of anthropogenic features because of the likely importance of bait abandonment and vehicular transport in earthworm spread. If roads are the point of introduction and facilitate subsequent diffusive spread (i.e. spread in a continuous manner away from an initial point), I hypothesized that distances along road networks would be a better predictor of genetic similarity than geographic distance. I also examined the extent to which genetic structure was influenced by whether a population occurred near a boat launch versus a road, or near an older road versus a newer one. I predicted that populations near boat launches would be

more genetically diverse than populations near roads, due to earthworm introduction from bait abandonment in addition to vehicle transport. Boat launches also receive more intense human use resulting in a greater number of vehicles occurring there over longer periods of time. As well, I predicted that the boat launch populations would be more genetically similar to each other than to the road populations. Road age was expected to have an effect similar to that of boat launches because older roads would likely have experienced a higher cumulative level of human activity, thereby resulting in more opportunities for multiple earthworm introductions at old roads relative to young ones. The particular haplotypes being introduced were also predicted to vary over time, leading to differing genetic composition at old versus younger roads.

4.2 Materials and Methods

4.2.1 Earthworm collection

Earthworms were sampled at 117 sites near boat launches and roads in 2005-2006 as described in Chapters 2 and 3 (Figure 4.1). Earthworms were collected underneath logs as well as from leaf litter quadrats. 60 of these sites had earthworms present, and I obtained sufficient numbers of *D. octaedra* specimens to conduct genetic analyses for 25 of these locations. Sites were located a minimum of 1.5 km apart. All earthworms living in physical contact with each other were considered to belong to a population, such that each site contained one population of *D. octaedra*. In other words, a population was defined as a group in which every individual is a potential common ancestor to the entire population at some point in the future (Templeton 1989). Earthworms were stored in 70% ethanol until DNA extraction. Species identification was verified using morphological characters outlined in Reynolds (1977).

4.2.2 Polymerase chain reaction and DNA sequencing

I extracted total genomic DNA from 10 earthworms per site using a Qiagen DNeasy Tissue Kit following the manufacturer's protocol. Universal primers (HCOI/LCOI) from Folmer *et al.* (1994) were used for PCR amplification and sequencing of a portion of the mitochondrial cytochrome *c* oxidase subunit I gene (COI). PCR reactions were carried out in a 50ul volume containing 2.5 mM $MgCl_2$, 0.8uM of

each primer, 0.2mM of each dNTP, 3U Taq, 20-50ng DNA, 19ul water, and 5ul PCR buffer (10mM Tris pH 8.8, 0.1% Triton X-100, 50mM KCl, and 0.16mg/mL bovine serum albumin). I ran the reactions on an Eppendorf Mastercycler ep using the following temperature cycling profile: 95°C for 2 minutes; 35 cycles with 95°C for 1 minute, 49°C for 1 minute and 72°C for 1.5 minutes; 72°C for 7 minutes; and a final hold temperature of 4°C. PCR products were purified using 1% agarose gel electrophoresis and a QIAquick Gel Extraction Kit. The gel-purified PCR products were sequenced in both directions using an ABI BigDye Terminator kit (Applied Biosystems). Reactions were carried out in 10ul volumes containing 2uM of each primer, 1.75ul Big Dye buffer, 0.5ul Big Dye RR mix, and 50ng DNA template. An Applied Biosystems 3730 DNA Analyzer was used for sequencing.

4.2.3 Data analysis

I aligned sequences using GeneTool 2.0 (BioTools Incorporated, Edmonton, Alberta), and identified unique haplotypes in ClustalW (Chenna *et al.* 2003) and Geneious 2.5.3 (Drummond *et al.* 2006). The haplotype diversity (h) of each population was calculated using ARLEQUIN version 3.1 (Nei 1987; Excoffier *et al.* 2005). To assess relationships between haplotypes, a haplotype network was constructed using the statistical parsimony method in the program TCS (Clement *et al.* 2000).

Population structure was assessed by performing analyses of molecular variance (AMOVAs) using the Jukes and Cantor model as implemented in ARLEQUIN version 3.1 (Excoffier *et al.* 1992; Excoffier *et al.* 2005). Other models of nucleotide substitution were tested, but produced similar results. In the AMOVAs, Φ-statistics were calculated to compare genetic differentiation within populations, among groups of populations, and across all populations. Φ-statistics are analogous to F-statistics and account for both haplotype frequencies and molecular distances between haplotypes. I performed one AMOVA in which there was one hierarchical group of all the populations and two AMOVAs in which populations were divided into groups based on whether they were adjacent to a boat launch or a road, or on whether they were near a young or older road. Approximate road ages were determined by reviewing aerial photos from Air Photo Distribution in Edmonton, Alberta. Roads classified as old ranged from 37 to 57

years old, while young roads were between 7 and 28 years old. Statistical significance was assessed by performing 1000 permutations of the data.

I compared the pairwise distances between populations (Φ_{ST}) obtained from the AMOVAs with geographic distance and distance along roads between populations using Mantel and partial Mantel tests in ARLEQUIN (Legendre and Legendre 1998). In ArcGIS 9.1 (ESRI Inc., Redlands, California), distances along road networks between populations were calculated using the Network Analyst extension (mean distance = 361.3 km) and geographic distances were calculated using Hawth's Analysis Tools for ArcGIS (mean distance = 227.6 km) (Beyer 2004). To test whether the number of haplotypes present in a population was affected by whether the population was adjacent to a boat launch versus a road, I used a t-test and Monte Carlo simulation. The effect of road age (old or young) was tested in the same way.

4.3 Results

A 609-bp fragment of cytochrome oxidase I was sequenced for 250 individuals. The percentage of variable sites was 10.67% and a total of sixteen haplotypes were detected (GenBank Accession nos: EU035477-EU035492; Table 4.1). Haplotype diversities within populations ranged from 0 to 0.644, with 11 of the 25 sites having a haplotype diversity of 0 (Table 4.2). Eight of the haplotypes were rare and found in only a single population, while the remaining haplotypes were shared among at least two populations. Haplotype 3 was the most common haplotype (f = 0.384), occurring at 13 of the 25 sites. Four highly divergent groups of haplotypes were evident in the 95% parsimony haplotype network (Figure 4.2). In the network, one group included 12 of the haplotypes, while the remaining three groups each consisted of only one or two haplotypes.

The AMOVA which included one hierarchical group detected a significant overall Φ_{ST} among populations (0.717, $p < 0.001$) (Table 4.3). Significant population differentiation was present, with the majority of genetic variation occurring among populations (71.7%) rather than within populations (28.3%). In both cases, the group-level (boat launch versus road sites and old versus young sites) accounted for very little of the genetic variation. Results obtained by calculating F_{ST} values were similar to those

reported using Φ_{ST}. There was also no evidence of a relationship between genetic variation and habitat grouping (aspen versus mixedwood forests).

The Mantel tests demonstrated that there were no significant relationships between genetic distance and geographic distance ($r = -0.089$, $p = 0.793$), or between genetic distance and road distance ($r = -0.069$, $p = 0.742$). When controlling for geographic distance, road distance was still not significantly correlated with genetic distance (partial $r = 0.018$, $p = 0.433$). Similarly, when controlling for road distance, geographic distance and genetic distance were not correlated (partial $r = -0.059$, $p = 0.713$). The average number of haplotypes present at boat launches was significantly greater than the number present at road sites ($p = 0.025$). However, road age did not significantly affect the number of haplotypes present within a population ($p = 0.382$).

4.4 Discussion

The amount of genetic diversity found in *D. octaedra* in northern Alberta was relatively high, similar to the results of allozyme studies conducted on this species (Hansen *et al.* 2006; Terhivuo and Saura 2006). Over half of the populations I sampled contained two or more haplotypes, and were likely founded by multiple invaders, from either single or multiple sources. The remaining populations each contained only one haplotype, which suggests that they may have been founded by a single individual. My results indicate that multiple introductions of earthworms have likely occurred across northern Alberta, but that individual populations may have been established by a single individual or multiple individuals introduced on either one or more occasions.

Significant population differentiation existed within my study area, a finding which further supports the conclusion that a number of discrete introduction events have occurred in this region. Occurrence of bottlenecks or genetic drift in some populations could have contributed to differentiation by reducing the number of haplotypes present in a population. However, bottlenecks are less common in asexually reproducing species than in sexually reproducing ones (Brown and Marshall 1981). Adaptive evolution following the initial colonization could also promote population-level genetic structuring. Three populations (C, I, T) did each contain the same pair of haplotypes (H6, H7) which differed by only a single base change. The transforming mutation may

therefore have occurred at one of those populations, which then could have acted as a source for spread to the other sites. It seems more likely that the mutation occurred in a source population outside of the boreal forest however, as the earthworm populations in my study were likely founded relatively recently. Road access to my sites, and hence most human activity, has only been present within approximately the last 60 years. Although there has likely been minimal adaptive evolution within this short time period, the population differentiation present in northern Alberta may allow for local adaptation of populations to their environments in the future. While parthenogenetic species are often viewed as evolutionary dead ends (Maynard Smith 1978; Kondrashov 1993), they are capable of rapid adaptation (Sunnucks *et al.* 1998; Simon *et al.* 2002). Parthenogenesis allows for large numbers of offspring to be produced, some of whom are likely to have mutations that are beneficial (Simon *et al.* 2002). Furthermore, recombination does not disrupt coadapted gene complexes in such species, and large population sizes can lead to more rapid responses to selection.

Four divergent groups of haplotypes were present within the populations, and there were no discernable morphological differences between them. The percentage of variable sites was high (10.7%) as compared to levels in other invasive invertebrates sampled at similar or larger spatial extents, such as the Colorado potato beetle (3.8%) and the mollusc *Cyclope neritea* (6.8%) (Grapputo *et al.* 2005; Simon-Bouhet *et al.* 2006). However, this degree of intraspecific variability appears to be fairly common within earthworm species. For example, in *Octolasion tyrtaeum*, a parthenogenetic earthworm species, 19.5% of sites in the cytochrome oxidase II gene were polymorphic (Heethoff *et al.* 2004). Similarly, alignment of the four cytochrome oxidase I gene sequences available in GenBank for *L. rubellus*, a sexually reproducing earthworm species, revealed that 24.6% of the sites were variable (Accession nos: DQ092901-DQ092904).

There was no significant relationship between geographic and genetic distances between populations. A pattern of isolation-by-distance would be expected if earthworms were spreading in a diffusive manner via active dispersal or via non-anthropogenic passive dispersal (eg. by birds, mammals, or wind). Instead, earthworms seem to be spreading via a series of human-mediated long distance jump dispersal

events, which is consistent with the idea that their ability to disperse independently is limited (Marinissen and van den Bosch 1992; Sakai *et al.* 2001). Although vehicles are expected to be a major vector involved in earthworm spread, genetic distance was not related to distance along road networks in my study. Jump dispersal from source areas to roads seems to be more common than diffusive spread along road networks within the boreal. There may also be many source populations, at locations such as towns, farms, boat launches, or other roads from which vehicles may transport earthworms. A more intensive examination of earthworm population genetics at a smaller spatial scale (i.e. along a road network with a single starting point) is needed to determine whether newly established populations of earthworms along roads are acting as sources for subsequent invasion events.

Boat launch sites had significantly more haplotypes present at them than road sites, which suggests that multiple introduction events may be most common near boat launches. This might be due to the introduction of earthworms at boat launches via bait abandonment, as well as by vehicular transport. *D. octaedra* is not normally used as bait but it is sometimes found in commercial bait along with the larger marketed species (Tiunov *et al.* 2006). Alternatively, there may have been a greater concentration of human activity around boat launches than roads, which would have allowed increased opportunities for introduction to occur. Boat launches and associated campgrounds are often the final destination of many recreational vehicles in the boreal forest. Vehicles may sit for longer periods of time at such sites, thereby increasing the likelihood of mud containing earthworms or cocoons falling from the vehicle. Different haplotypes do not appear to occur at boat launches as compared to roads. This suggests that similar haplotypes are transported via both vehicular transport and bait abandonment, or that few introductions are actually occurring via bait abandonment.

Because older roads may often experience a higher cumulative amount of vehicle traffic than roads built more recently, I hypothesized that there would be greater propagule pressure on older roads. This could have led to an increased number of introductions occurring along such older roads as compared to newer ones. In fact, as was reported in Chapter 3, road age did significantly affect the probability of earthworm occurrence in northern Alberta, with older roads having a higher frequency of occurrence

of earthworms than younger roads. Yet, road age had little influence on the number of haplotypes present at a site. It may be rare for introductions to occur at the exact same location along a road, meaning that additional introductions would not lead to increased numbers of haplotypes at a point location. Alternatively, this result may indicate that after some period of time, few additional haplotypes are likely to be introduced to a site because most of the haplotypes present at the source area(s) have already been introduced. Examining the relationship between road age and the number of haplotypes at younger roads might reveal such a threshold. It is also possible that cumulative traffic levels were not actually higher at the older roads, and therefore multiple introduction events would not have been more likely to occur at those sites than at the younger road sites. Genetic composition also did not differ between old and young roads, indicating that the haplotypes being introduced did not vary over time. This suggests that the source populations and mechanisms promoting spread have not changed substantially over time.

Although a number of invasion genetics studies have examined whether single versus multiple introductions have occurred for different species, genetic data has been less frequently used to distinguish between alternative introduction mechanisms or colonization scenarios. Examination of which environmental factors are important in determining whether individual populations are established by single or multiple introductions is one approach that may lead to greater insight into the importance of alternative introduction mechanisms. Information on which types of areas are most likely to have experienced multiple introductions may help to identify other populations that are likely to have increased genetic diversity and may act as highly diversified sources for future spread. It may also help to identify uninvaded areas that are most likely to be at high risk of future invasion. This could then allow management strategies to be developed to target those areas. For example, my results suggest that management strategies that are designed specifically to reduce earthworm introductions at boat launches (eg. by prohibiting bait abandonment) could be particularly beneficial.

In conclusion, multiple introductions of *D. octaedra* appear to have occurred in northern Alberta. The amount of genetic diversity was found to be relatively high, leading to an increased potential for adaptation, and possibly greater invasion success in

the future. The amount of diversity I found within this area suggests that the approach taken in many studies on invasive species, whereby sampling is done at only one or a few locations per state or country, may not be appropriate for all species. If *D. octaedra* had been sampled at only a few locations, it may have been concluded that one introduction event, rather than multiple introductions, had occurred. Boat launches had greater numbers of haplotypes present than roads, which suggests that multiple introductions were more likely to occur at those locations. Focusing management efforts on those areas which are most likely to experience multiple introductions could help to reduce the adaptive potential and invasion success of non-native species.

Table 4.1. Nucleotide sequences for unique haplotypes. The sequences at variable sites are shown for each unique haplotype. The entire complete sequence for H3 and the position numbers of the variable sites are shown below the table.

Haplotype	Sequence* at variable sites†
H3	CTGAACTCATTTCCCAATCTCACCGCAATATGCGGGTATACTCTCTGCGCCTAAAGATGTGATTG
H2	.....TAT..CC..A.....CC..A..AC.....T.....A....G....C.A....
H4	T.T.....CTT.....G.....T.....
H5	T.....C.....
H6	T.T.....C.....A.....A....
H7	T.....C.....G...A....
H8	T..G.....C.....T...GA....A....
H9	T..G...TG.CC..T....C.....T...C.A...A.....T.....A...CG...C..A....
H10	T.....C.....A.A.....A.....A....
H11	.....C.....
H12	T..G.....C.....A....
H13	T...C.....C.....A.C..
H14	.....TA...CC..A.....CC..A..AC.....T.....A....G....C.A....
H15	T....T.....C.....
H16	TC.....
H1	T.AGC....CCCTTAGGCG...TTA.GG.T..TAATCGCCTC.CTCATAC.C..GA..CCAC.CA

*H3 complete sequence:

CATTTGAGCTGGAATGGTAGGAGCAGGCATAAGATTACTAATCCGAATTGAACTTAGCCAACCCGGAGCATTTCTAGGAAGAGATCAAC
TATATAATACTATTGTAACAGCCCACGCATTTGTTATAATTTTCTTTTTAGTCATACCCGTATTTATTGGAGGATTTGGAAACTGACTC
CTTCCTCTCATACTAGGAGCACCTGACATAGCCTTTCCTCGACTAAATAATATAAGGTTTTGACTATTACCCCCATCCCTAATTCTTCT
TGTATCTTCAGCAGCTGTAGAGAAGGGCGCGGGAACGGGTTGAACAGTGTATCCACCTCTTGCAAGAAACTTGGCTCATGCTGGGCCAT
CAGTAGACTTAGCTATTTTCTCCCTTCACTTAGCTGGAGCCTCTTCAATTTTAGGTGCAATTAACTTTATTACTACAGTTATCAATATA
CGATGATCGGGACTACGGCTAGAGCGAATTCCCCTATTTGTCTGAGCTGTACTAATTACAGTTATTCTACTTCTCCTATCACTGCCTGT
ATTAGCGGGGGCAATTACTATACTTTTAACAGACCGAAATTTAAATACGTCATTTTTTGATCCTGCGGGAGGGGG

†Variable sites: 1, 4, 17, 19, 25, 38, 55, 58, 61, 73, 97, 103, 112, 115, 142,160, 163, 166, 178, 181, 187, 190, 191, 205, 235, 242, 244, 253, 274, 277, 283, 292, 295, 304, 314, 316, 319, 322, 343, 355, 364, 406, 421, 436, 439, 442, 454, 458, 463, 479, 497, 511, 514, 523, 526, 529, 535, 536, 541, 580, 583, 586, 595, 598, 607

Table 4.2. Numbers of each haplotype and haplotype diversity for each population. Populations located at boat launches are denoted with an asterisk.

	Haplotypes																
Populations	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	Haplotype diversity (h)
A	4	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.5333
B	–	–	9	1	–	–	–	–	–	–	–	–	–	–	–	–	0.2000
C	–	–	–	–	–	5	5	–	–	–	–	–	–	–	–	–	0.5556
D	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
E*	–	–	9	–	–	–	–	–	–	1	–	–	–	–	–	–	0.2000
F	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	0
G	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
H	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	0
I	–	–	–	–	–	9	1	–	–	–	–	–	–	–	–	–	0.2000
J	–	–	8	–	–	2	–	–	–	–	–	–	–	–	–	–	0.3556
K*	–	–	6	–	2	–	–	–	2	–	–	–	–	–	–	–	0.6222
L	–	–	–	–	–	10	–	–	–	–	–	–	–	–	–	–	0
M	–	–	–	–	–	–	–	–	–	–	–	3	7	–	–	–	0.4667
N	–	–	1	–	6	–	–	–	–	–	–	–	–	2	1	–	0.6444
O*	–	1	6	–	–	3	–	–	–	–	–	–	–	–	–	–	0.6000
P	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	0
Q	–	–	9	–	–	–	–	–	–	–	–	–	–	–	–	1	0.2000
R	–	–	–	–	–	–	10	–	–	–	–	–	–	–	–	–	0
S*	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	0
T	–	–	–	–	–	3	7	–	–	–	–	–	–	–	–	–	0.4667
U	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	0
V	–	–	–	–	–	–	–	–	–	–	–	10	–	–	–	–	0
W	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	0
X	–	–	1	–	–	–	–	–	–	–	–	–	–	9	–	–	0.2
Y*	–	–	7	–	1	–	–	1	–	–	1	–	–	–	–	–	0.5333

Table 4.3. Results of AMOVAs calculated using Jukes and Cantor distances among haplotypes. Analyses were conducted using one hierarchical group which included all populations as well as with an additional hierarchical group which divided populations into groups based on whether they were near a boat launch or a road, or whether the population was near a young or old road. An asterisk denotes statistical significance at $\alpha = 0.05$.

Hierarchy	Categories	% variation	Φ estimate
One group	Among populations	71.73	Φ_{ST}=0.717*
	Within populations	28.27	
Two groups (boat versus road)	Among groups	-0.94	Φ_{CT} = -0.00936
	Among populations within groups	72.49	Φ_{ST} = 0.715*
	Within populations	28.45	
Two groups (old versus young)	Among groups	-4.52	Φ_{CT} =-0.0452
	Among populations within groups	75.73	Φ_{ST} = 0.712*
	Within populations	28.78	

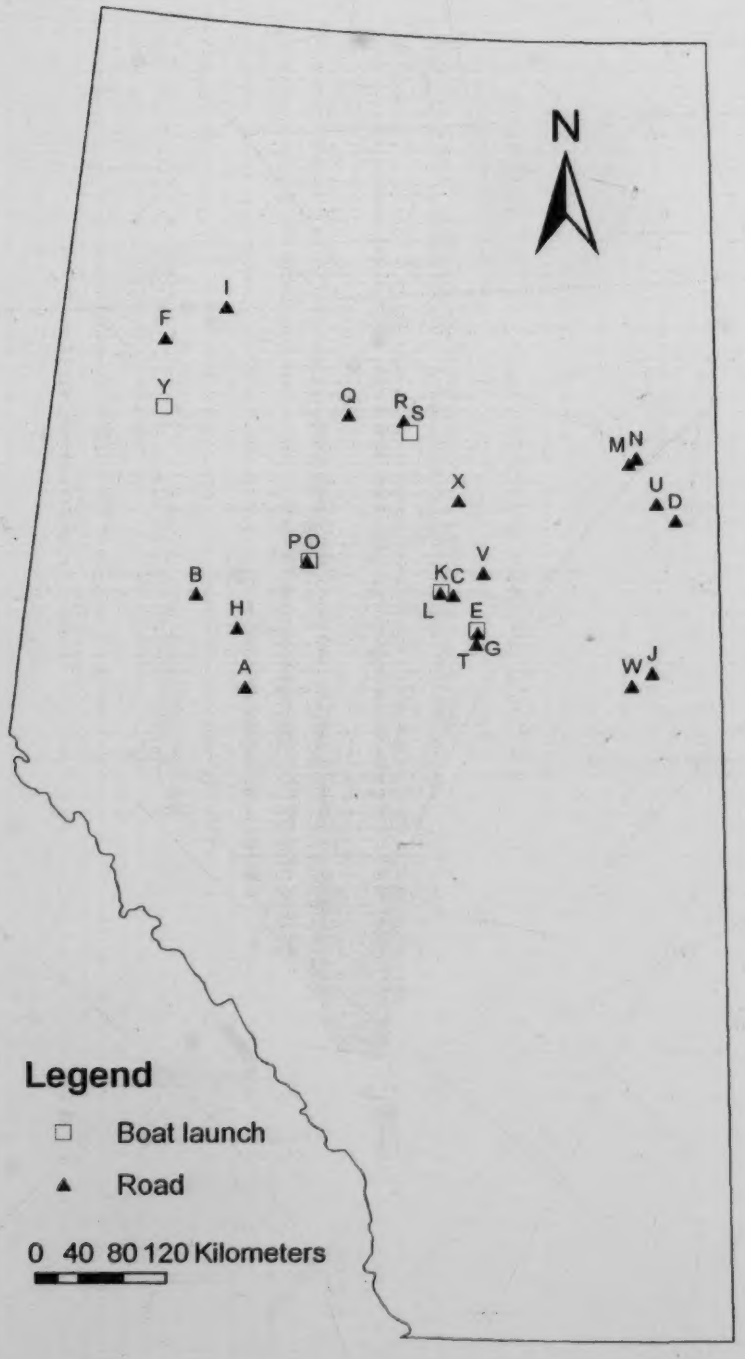

Figure 4.1. Locations of study sites (n=25) within the boreal forest of northern Alberta (shaded in gray). White squares represent sites near boat launches and black triangles represent sites near roads.

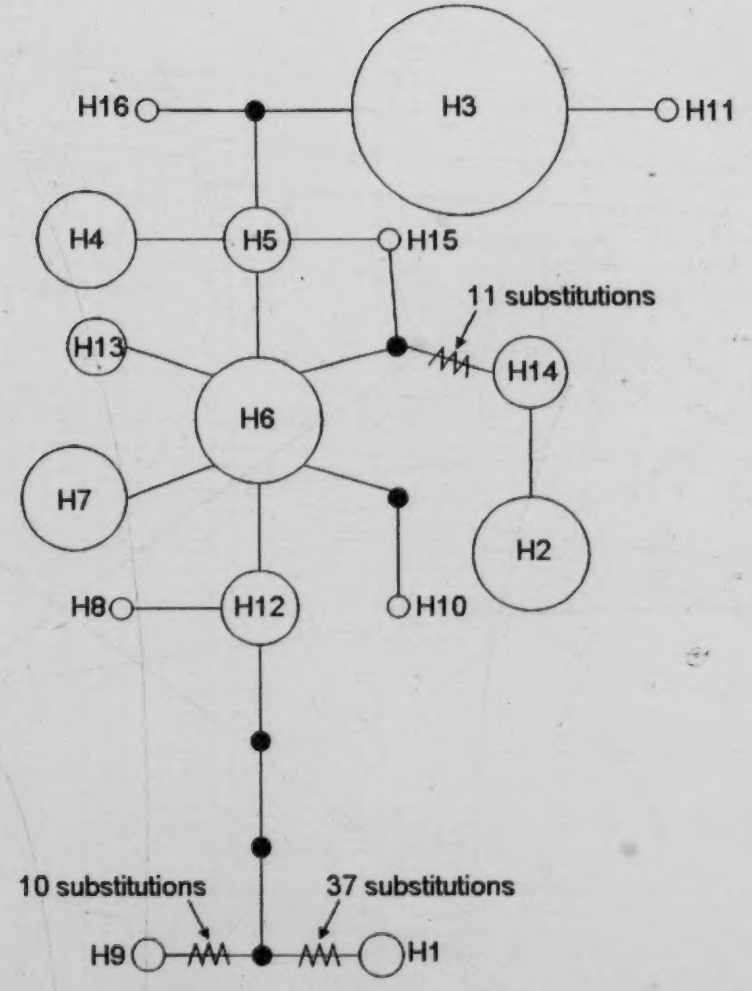

Figure 4.2. Mitochondrial haplotype network. The areas of the circles are proportional to the number of samples of each haplotype. The lines represent single nucleotide mutations and black circles represent haplotypes not observed in my study.

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CHAPTER 5. SYNTHESIS AND MANAGEMENT IMPLICATIONS

5.1 Research summary

The purpose of this thesis was to investigate how earthworms are introduced and spread in Alberta's boreal forest. Specifically, I found that earthworms were more likely to occur near boat launches and roads than seismic lines, forest interiors, or far shores. My results indicate that bait abandonment and transport by vehicles are likely the major mechanisms involved in earthworm introduction in boreal Alberta. Introduction along roads might occur either via transport of earthworms or cocoons in soil or gravel used for road construction, or via transport of cocoons in tire treads or elsewhere on vehicles. However, earthworms were more likely to be present at older roads than younger roads, which suggests that introduction during construction is a less likely explanation than later introduction via vehicular traffic. Earthworms had also spread over significantly greater distances at older roads than younger roads. Using this data, I created a model which suggested that earthworms likely occupy approximately 9% of suitable forest habitat in the Al-Pac FMA of northeastern Alberta at the present time. If current rates of development continue, this is projected to increase to 49% of suitable habitat in the next 50 years. Overall, these results emphasize the importance of landscape structure and linear features in promoting the spread of invasive species. Consideration of landscape structure may be critical for the construction of realistic models of invasive spread (With 2002).

I also found that multiple introductions of *D. octaedra* are likely to have occurred in northern Alberta. There was no relationship between genetic distances and either geographic distances or distances along road networks, suggesting that human-mediated jump dispersal is more common than diffusive spread via road networks or via active dispersal. Haplotype diversity at sites adjacent to boat launches was significantly higher than at sites located near roads alone, suggesting that multiple introductions may be most likely to occur in these locations. Population genetics studies such as this can help to determine whether invasive species are established via single versus multiple introduction events, and can also be used to distinguish among various possible colonization scenarios.

5.2 Limitations and Future Research Needs

Anthropogenic activities appear to be the major vectors involved in earthworm introduction and spread in northern Alberta. However, other mechanisms not directly related to human activity may also be important. For example, further research on the roles of wind, non-human vertebrates (eg. birds), and streams in promoting earthworm spread could be useful. A wind tunnel could be used to assess whether cocoons or small earthworms can be dispersed by wind at speeds comparable to those encountered along linear features and in forest interiors. Linear features may act as a "funnel" for wind which could facilitate further spread along road networks once earthworms establish. Occurrence of internal transport by birds might be investigated by assessing the viability of cocoons after passage through birds' digestive systems. Finally, earthworm surveys near streams and collection of drift samples could permit an estimate of the extent to which earthworm and cocoon transport occurs along waterways. Streams may be particularly important for the dispersal of bait species that occur mainly near boat launch sites, as streams flowing out of lakes might allow rapid transport of these species to new areas. A key uncertainty is whether new dispersal vectors may become important in earthworm spread as earthworm density increases locally and at the landscape-scale.

Inspections of vehicles and construction equipment for earthworms and cocoons could help to clarify the role of vehicular transport in earthworm spread, as well as quantify the frequency of this type of transport. Additional research on the probability of earthworm occurrence near other types of anthropogenic features could also allow an increased understanding of earthworm spread. Well sites and pipelines are projected to become increasingly prevalent on the landscape in northern Alberta (Schneider *et al.* 2003), and thus may be important in earthworm introduction. More precise estimates of local spread rates should also be obtained by tracking the location of the invasion front at sites over multiple years. This information, along with additional data on occurrence near streams, pipelines, and well sites, would allow a more accurate spread model to be created. Also, my model calculations assumed that earthworms do not occur in black spruce and tamarack stands. Because earthworms are commonly found in conifer-dominated forests in Europe (eg. Terhivuo 1989), more sampling should occur in these habitats to verify that this assumption is correct.

There appeared to be no relationship between genetic distances in *D. octaedra* and distances along road networks. However, this research was conducted at a large spatial scale. A more intensive examination of earthworm population genetics at a smaller scale (i.e. along a road network with a single starting point) is needed to determine whether newly established populations of earthworms along roads are acting as sources for subsequent invasion events along a particular section of a road network. An investigation of the population genetic structure of other non-native earthworm species in northern Alberta, particularly a species that reproduces sexually rather than via parthenogenesis, could also be informative.

A key unanswered question is whether the effects of earthworm invasions in the boreal forest are similar to those found in other forested ecosystems. Earthworm abundances do appear to be comparable to those in other locations, as discussed in Chapter 2. However, epi-endogeic, endogeic, and anecic earthworms, which typically have the largest impacts on invaded systems, appear to be less common in the boreal than in the United States and southern Alberta (Gundale *et al.* 2005; Eisenhauer *et al.* 2007). This is likely due primarily to the fact that invasions in northern Alberta began later than in other areas where human settlement occurred earlier. The different soils, climate, and vegetation types found in northern boreal forests might also be an important factor. For example, the high clay content of typical boreal soils may negatively impact survival of mineral soil-dwelling species. However, climatic and soil conditions in the foothills of southern Alberta are somewhat similar to those found in the boreal forest. Additionally, my sampling methods may have resulted in an underestimation of abundances of non-epigeic species. Mustard extraction or sorting of deeper mineral soil layers may be a more effective sampling method for some of these species (Hale *et al.* 2005). Research to elucidate the effects of non-native earthworms in the boreal forest should examine their impacts on leaf litter decomposition, soil structure, nutrient cycling, bacteria, fungi, plant communities, and vertebrates.

5.3 Management Implications

Although the consequences of earthworm introductions in the boreal forest remain unclear, the significant impacts they have had in other forest systems suggest that

measures must be taken to reduce future introduction and spread. The importation of earthworms into Canada is currently regulated (Canadian Food Inspection Agency 2006). However, this regulation exists due to concern that plant pests may be present in soil associated with earthworms, rather than concern about the invasiveness of earthworms themselves. In fact, any earthworm species that is known to occur in Canada may be imported from the continental United States and *L. terrestris* may also be imported from the Netherlands. Because most of the invasive earthworm species present in the United States have also been reported in parts of Canada, the effectiveness of this regulation for reducing earthworm invasions is likely limited.

My research indicates that reducing vehicular transport and bait abandonment may be especially important for earthworm control in the boreal forest. Reducing construction of new roads, restricting the amount of traffic on roads, or reclaiming temporary roads could help to reduce vehicular transport of earthworms and cocoons. Because the species of earthworms that are commonly used as bait have had the largest impacts on forest systems in other areas (eg. Gundale *et al.* 2002; Hale *et al.* 2006), a priority should be placed on introducing measures to limit bait abandonment. At a minimum, Alberta should follow the example of jurisdictions, such as Minnesota, which have laws prohibiting bait abandonment (Office of Revisor of Statutes, State of Minnesota 2006). Public education must also be a critical component of any earthworm management effort (Callaham Jr. *et al.* 2006). One such program to educate the public about the ecological consequences of earthworm introductions in Minnesota has been well-received (Callaham Jr. *et al.* 2006).

Earthworms are only one group out of a number of species that are currently invading Canada's boreal forest. The rapidly accelerating levels of industrial and recreational development in this region suggest that species invasions will become an increasingly important issue. Consequently, it is critical that we introduce management strategies to limit the introduction and spread of invasive species in the boreal.

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